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OBSERVATIONS ON HEMODYNAMIC FACTORS AND LEFT HEART PERFORMANCE IN ESSENTIAL HYPOTENSION

BY

M. H. FRICK



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From the Wihuri Research Institute
Helsinki, Finland

OBSERVATIONS ON HEMODYNAMIC FACTORS AND LEFT HEART PERFORMANCE IN ESSENTIAL HYPOTENSION

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PREFACE

To Professor Pentti I. Halonen, M.D. head of the Wihuri Research Institute and of the First Medical Clinic, University of Helsinki, I wish, first of all, to express my deep feeling of gratitude. He suggested the topic and followed my work with untiring interest. His constructive criticism and advice was of decisive importance in approaching the problems.

I am greatly indebted to Docent Martti J. Karvonen, Ph.D. (Oxon.) head of the Physiological Department of the Institute of Occupational Health, Helsinki, for encouraging interest and advice through the various stages of my work.

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I am grateful to Professor Lars Werkö, M.D. head of the First Medical Service Sahlgrenska Sjukhuset, Göteborg, for authoritative counsel in connection with my technical problems.

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The statistical treatment of the results was carried out by Mrs. S. Asp, M.A., and Mr. E. Järvinen, M.A., whom I hereby wish to thank.

For pleasant co-operation in checking the manuscript I am grateful to Miss Elvi Kaukokallio.

My heartfelt thanks are also due to my wife for her untiring support and patience through all phases of my work.

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Helsinki, January 1962

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Vammala 1982
Vammalan Kirjapaino Oy

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In several disease states low blood pressure, hypotension, is an outward reflection of hemodynamic alterations. It is due to either an inadequate cardiac output, insufficient peripheral resistance, or a reduced circulating blood volume as the primary cause. In these connections hypotension is a secondary phenomenon, curable together with the primary disease.

Two subdivisions under the caption of hypotension, i.e., orthostatic and postural hypotension, have received attention, since they can be handled as their own entity. Orthostatic hypotension is characterized by following signs manifested on changing from recumbency to an upright position. The systolic blood pressure falls considerably whereas the diastolic pressure either remains unchanged or is slightly levated. The pulse rate usually increases by at least 27 beats per minute. Pathogenetically orthostatic hypotension is a failure of the compensatory adaptive mechanisms operative to maintain the blood pressure level in the erect position. In spite of the reflex acceleration of the heart rate cardiac output falls because of a reduced venous return due to pooling of blood in the splanchnic area and the lower extremities.

While the blood pressure regulating mechanisms responsible for the maintenance of the blood pressure in the erect position are inadequate in orthostatic hypotension, they are completely lacking in postural hypotension. An upright position causes a fall in both the systolic and diastolic blood pressures without an acceleration response in the heart rate. The cardiac output is reduced in the same way as in orthostatic hypotension. Neuropathological conditions either in the hypothalamic region, in the medulla, or high in the spinal cord are held responsible for postural hypotension.

The circulatory adjustment in the two above cited forms of hypotension has been extensively studied and is fairly well understood. On the other hand, information concerning the circulation in persistently low arterial pressure is quite meager and contradictory. This is due at least in part, to investigators neglecting to accept the consistently low arterial pressure as its own entity and taking cognizance of the blood pressure distribution only with regard to normotension and hypertension.

The present investigation is an attempt to approach the hemodynamics of essential hypotension both at rest and during physical exercise.

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sure as an upper limit of hypotension, the following figures were obtained from some earlier studies (Alvarez, Wulzen & Mahoney 1923 Diehl & Sutherland 1925 Robinson & Brucer 1939) representing a total of 15164 men and 16682 women. For men the upper systolic limit of hypotension in the age of 20—40 was from 98 to 107 mm. Hg The figures for females were in general about 2—5 mm. Hg lower throughout

Perhaps the most extensive study ever performed on the subject of blood pressure in a general population is that of Bøe Humerfelt & Wedervang (1957) In connection with a mass x ray and tuberculosis examination, blood pressure readings were obtained from a total of 27718 males and 40258 females in Bergen, a city with a population of 112000 inhabitants. The figures represent 70 per cent and 80 per cent, respectively of all adults in the city In addition to this enormous material the study has the further merit of an extraordinarily complete statistical treatment The investigation was carried out in two stages, and the material was accordingly divided int two groups giving slightly varying results It may suffice in this connection to cite the data for the first group (26281 females and 17901 males) Applying the same rule of -2 standard deviations from the mean blood pressure as the upper limit of hypotension the following conclusions can be drawn For men this demarcation was a systolic blood pressure of 106 through the age of 20 to 39 Thereafter the limit showed a slight rise from 103 to 107 ending at 110 at the age of 70—74 years. In the female population the limit moved from 100 at the age of 20 to 102 at the age of 40—44. Beginning with the age of 45—49 it rose steadily to a value of 118 mm. Hg at the age of 70—74 years.

In contradistinction to essential hy pertension, the level of the diastolic blood pressure has been regarded as an insignificant determinant of essential hypotension. Accordingly the brief survey given above of the statistical approach to the limits of hypotension relates only to the values of the systolic blood pressure.

In practice the exact figures obtained from statistical studies have been rounded to 110 mm. Hg (e.g., Warburg 1943 Best & Taylor 1945 Siedek, Wenger & Hörtnagel 1950 Aalamer 1951, Löffler 1954 Pennock 1957) to 105 mm. Hg (e.g., Martini & Pierach 1926 Laberke 1952, Pellegrini 1952) or to 100 mm. Hg (e.g., Silvestrov 1956) of systolic blood pressure for men. Approximately 5 mm. Hg lower values have been applied in selecting female subjects. The value of 110 mm. Hg is placed in the zone of bor derline hypotension of Master Dublin & Marks (1950) and the lower values are classified with greater cer tainty in the statistical group of real hypotension. It must, nevertheless, be kept in mind that both the values used in practice and the values obtained from statistics are arbitrary ones, because no sharp division can be made between clearly normal and clearly abnormal blood pressures.

SURVEY OF LITERATURE

Limits of hypotension

The question of the upper limit of hypotension is closely related to the definition of normal blood pressure Consequently data revealing the nor mal range of blood pressure are the source of our knowledge of the blood pressure figures that indicate a low arterial pressure Many statistics have been published to clarify the normal range of blood pressure Unfortunately with regard to hypotension, the majority of these studies have been concentrated on the establishment of criteria for normal blood pressure *contra* hypertension. Especially much of the material collected by life insur ance companies has been analyzed chiefly to clear up this demarcation (Fisher 1914 Symonds 1923)

A much quoted and greatly valued study is that of Master Dublin & Marks (1950) The statistical treat ment used in this study offers a possibility for the retrospective evaluation of some earlier reports. Using the standard deviation (S.D) as a yardstick, the blood pressure values were arranged into a central 80 per cent (slightly extended ± S.D) representing the normal range, and the values outside ± 2 S.D., in practice 95 per cent, represented hypertension and hypotension. The areas between the limits of normal and the limits of probably abnormal were narrow borderline zones. The final tabulated data included 7722 men and 7984 women from eleven industrial plants and from army airfields. The values presented for this series were as follows· The upper limit of hypotension for men was a systolic pressure of 98 mm. Hg at the age of 20—24 with a rise to 102 at the age of 35—39 and to 108 mm. Hg at the age of 60—64 years. *The corresponding normal ranges were* 105—140 110—145 and 115—170 mm. Hg, respectively The limits of hypotension for women were a systolic pressure of 95 at the age of 20—24, with a rise to 100 and 105 respectively in the same age groups as for men. The normal ranges for systolic blood pressure in the above mentioned age groups were 100—130 105—140 and 115—175 respectively

A close agreement with the above mentioned values for men was found by Hamilton *et al.* (1954) in a study of 1204 female and 827 male patients treated in clinics for diseases not con nected in any way with hypertension. In the case of females, somewhat high er systolic pressures were found.

In retrospect, using the -2 standard deviations from the mean blood pres-

tigations on hypotensive subjects in the supine position revealed in the majority of cases an increased peripheral resistance and reduced cardiac output. This low output was further reduced in the standing position, with an increase in the peripheral resistance. While the findings in these two body positions showed some divergence, more uniform results were obtained during exercise on a bicycle ergometer. The stroke volume, determined at the third minute of exercise, increased less than in trained subjects. The less than normally increased cardiac output was thus mainly due to an increase in the pulse rate. The recovery of the pulse rate and cardiac output to normal after the exercise period was clearly delayed. Reindell stated that a blood pressure of 95—100 mm. Hg is to be regarded as hypotension. However figures such as 115/80, 140/90 and 117/75 in supine rest are to be found in the tabulated data of his patients. The statements that the blood pressure is often normal at rest and that the abnormal regulation manifests itself first in the standing position or after exercise give reason to believe that the material was not solely composed of essential hypotension in sensu stricto but included also cases of orthostatic and postexertional hypotension. In addition, all the patients were asthenic individuals, some of them in the extreme, with a poor physical fitness and other stigmas of asthenia.

Aalsmeer (1951) attached importance to a feature often seen in hypotension, namely to the small pulse pressure due to a reduced systolic pressure in association with a normal diastolic pressure. The same phenomenon was more closely studied by Luisada (1929, 1948) who called it hyposphygmia. Characteristically the pulse pressure at the arm is very small (e.g., 105/90) but an extreme reduction of the pulse pressure is found at the forearm, with figures of 95/90 resulting in many cases in unmeasurable pulsations at the radial artery. The same tendency to a further reduction of the pulse pressure peripherally is also present in the lower extremities. The syndrome is further characterized, according to Luisada, by a constant orthostatic tachycardia and hypotension. The x-ray reveals a small, vertical heart. Poorly developed muscular tissue of the arterial walls, with possible secondary changes evoked by infection and intoxication, together with a reduced systolic discharge of the heart in some cases, was suggested by Luisada as possible explanations.

Sledek, Wenger & Hörtnagel (1950) studied 60 females and 40 males with an upper systolic blood pressure of 105 mm. Hg and 110 mm. Hg, respectively. Physical methods were used to obtain the cardiac output (Broemser & Ranke 1933, Böger & Wezler 1937). The first observation worth being noticed was the distribution of the series with regard to body build. Forty-five per cent of the males were asthenic individuals, 32 per cent had a normal body build, 12 per cent were athletes and 10 per cent had a pycnic build. The corresponding figures for females were 44, 35, 7 and 14 per cent.

Secondly in view of the many factors known to influence blood pressure for example race climate nutrition occu pation and body build the various statistics hardly are directly comparable

Earlier studies

In 1903 Ferrannini described a syn drome which he called Angioipotonia constituzionale This syndrome was characterized by low arterial blood pressure asthenic habitus, pendulous heart, pallid cyanosis of the skin and mucous membranes, bradycardia, dizziness, and frequent acrocyanotic manifestations. At that time no objective method was available to unravel the complicated question of hemodynamics in this disorder Later on, the question of hemodynamics and eti ology of hypotension retained its popularity and numerous reports were published on this topic. In 1926 Mar tini & Pierach summarized the evi dence published up to that time and presented their own investigations. The available data included six theories for the reduced blood pressure 1. Weak ness of the heart muscle 2 abnormal high resistance of the ascending aorta, 3 abnormally elastic large and medium sized arteries, 4 abnormal low resist ance in small arteries, 5 a reduced total blood volume and 6 a reduced blood viscosity These authors own series included 18 patients with an upper systolic limit of 105 mm. Hg for men and 100 mm. Hg for women. This limit was selected because subjects exhibiting higher blood pressure had not symptoms enough to be suit able for the study Blood pressure was recorded with a sphygmomanometer and the mean pressure was cal culated by halving the sum of systolic and diastolic pressures. Stroke volume was determined by the ethyl iodide method of Henderson & Haggard (1925) and the heart work was obtained by multiplying the stroke volume, heart rate and mean blood pressure as water The size of the heart was determined by measuring the transversal diameter in chest x rays. Peripheral circulation was evaluated by measuring the venous and capillary pressures. The following principal findings were obtained. Nine of the 18 patients were bradycardic, but in the whole series the pulse rate was observed to have a considerable range 45—107 beats per minute. The transversal diameter of the heart was in general 1—3 cm smaller than the corresponding figure in normal subjects. The stroke volumes were within the normal range in 14 cases and clearly reduced in four cases. The mean pressure and heart work were low Data on the peripheral circulation were suggestive of a reduced resistance On the basis of these results the authors concluded that hypotension can be traced back to either an impaired heart work or dilated smaller arteries.

With regard to peripheral resistance Reindell (1949) drew conclusions that were quite the opposite to the above The sphygmographic method of Broemser & Ranke (1933) was used to determine the cardiac output. Inves-

of the cardiac output gave too high values throughout. The cardiac output obtained by Fick was therefore reduced by a correction factor derived from the values obtained by the physical method of Böger & Wezler. The total blood flow was found to be within normal limits. The mean for the 17 tabulated patients was a cardiac index of 2.50 l. min. This figure for the normals was 2.64 L/min. Also the stroke volumes were within normal limits. The total blood volume, determined by a dye method, was 85.2 ccm. kg., compared with a mean value of 82.3 ccm. kg. of the controls. Nothing abnormal could be found in the circulation times in the lesser and greater circuits. The heart volume was determined according to Jonsell (1939) and the following values were obtained. Hypotensive subjects, mean 640 ccm., range 510—820 ccm. control subjects, mean of 740 ccm range 625—850 ccm. When the heart volume was divided by the body weight no significant difference was found. Finally the pulse rate at rest was quite normal and no tendency towards a vagotonic pulse was observed.

The syndrome of arterial hypotension, a term used by Pellegrini, was defined as a state of decreased tone of the smaller arteries and arterioles, resulting in a reduced peripheral resistance.

Silvestrov (1956) used sphygmographic methods for determination of the hemodynamic indices in hypotension. His 36 subjects were arranged into three groups, defined as healthy subjects with a low arterial pressure (below 100/60 mm. Hg) patients with symptomatic hypotension, and patients with the Da Costa syndrome and low blood pressure. All the groups showed an elevated cardiac output and a greatly reduced peripheral resistance which was regarded as the principal cause of the low arterial pressure.

In hemodynamic terms, blood pressure is a reflection of cardiac output and peripheral resistance. The results obtained by the authors quoted above are quite contradictory with respect to these basic parameters. Using exactly the same method, Reindell (1949) and Silvestrov (1956) reached opposite results. Reindell's observations were confirmed by the study of Swedek, Wenger & Härtnagel (1950) whereas Pellegrini's group attached importance to an abnormality of the peripheral circulation. These divergent results can hardly derive their origin from methodological differences, although full reliance cannot be given to indirect physical methods for determining the cardiac output (Hamilton 1945). It is more likely that the composition of the case series is to be held responsible for the variance in the results. All except Thacker (1940) studied patients with a variety of symptomatology which was a prerequisite for a closer hemodynamic evaluation. Even if much importance was not attached to the relationship between the symptoms and the hemodynamics, it is evident that a majority of the patients examined were selected by paying special attention to symptoms indicating disturbances in the

The pulse wave velocity a reflection of the distensibility of the arterial system, was found to be low in several cases. The windkessel was shortened in 80 per cent. The cardiac output was reduced in 44 per cent of the cases, the reduction being marked in 28 per cent. The peripheral resistance was normal in a majority of the subjects. Measurement of the oxygen debt after an exercise period at a uniform load of 1200 watts revealed clearly increased values of oxygen consumption in approximately 68 per cent of the whole series. Twenty-eight per cent of the males and 35 per cent of the female subjects showed an orthostatic pulse and blood pressure reaction. The authors made an attempt to divide the cases into groups since the parameters measured showed considerable variation from case to case. Two main groups could be distinguished a) a vagotonic type with good physical fitness, and b) a neuroendocrine type with possible disturbances in the pituitary-adrenal system.

Thacker (1940a) performed cold pressor tests (Hines & Brown 1936) on 55 hypotensive 96 hypertensive and 128 normotensive students. The following results were obtained. Hypertensive and hypotensive classes reached the maximum increase in the systolic and diastolic blood pressures more slowly than the normotensives the same slow reaction was found on returning to the basal level. The systolic rise was 10 mm. Hg in normals 11 mm. Hg in hypotensives and 18 mm Hg in hypertensive subjects. The corresponding rise in the diastolic blood pressure was 14 mm. Hg, 11 mm. Hg and 16 mm. Hg, respectively The same author (1940 b) studied also the response of the pulse rate and blood pressure to an exercise test consisting of raising the weight of the body from the floor onto a chair 18 1/2 inches high at a rate of 40 cycles per minute No difference in the return of the pulse rate to the basal level after the exercise was observed between normal and hypotensive subjects. The average increase of the systolic blood pressure evoked by exercise was 37 mm. Hg in the normals and 23 mm. Hg in hypotensive subjects. The diastolic pressure decreased in controls by 12 mm. Hg and in hypotensive subjects by 13 mm. Hg.

In 1952 and later in 1955 Pellegrini summarized the results obtained by him and members of his staff (Bobba 1952 Malamani 1952 Malamani & Capra 1952 Pellegrini Piotti & Baldrighi 1952, Martignoni, Piotti & Drovanti 1953 Bobba & Baldrighi 1954 Baldrighi 1955 a, b, c, d Cel Malamani & Tronconi 1955 Malamani 1955 a b Malamani & Mura 1955) The subjects studied by these authors were defined as patients exhibiting persistently low systolic and diastolic pressures without a reduction in the pulse pressure. Using the heart catheterization technique the pressures of the right heart and pulmonary artery were found to be within normal limits or occasionally elevated. The left ventricular pressure was correlative to the low systemic arterial pressure In the experience of the authors the direct Fick method for determination

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The pulse wave velocity a reflection of the distensibility of the arterial system was found to be low in several cases. The windkessel was shortened in 80 per cent. The cardiac output was reduced in 44 per cent of the cases, the reduction being marked in 28 per cent. The peripheral resistance was normal in a majority of the subjects. Measurement of the oxygen debt after an exercise period at a uniform load of 1200 watts revealed clearly increased values of oxygen consumption in approximately 68 per cent of the whole series. Twenty-eight per cent of the males and 35 per cent of the female subjects showed an orthostatic pulse and blood pressure reaction. The authors made an attempt to divide the cases into groups since the parameters measured showed considerable variation from case to case Two main groups could be distinguished a) a vagotonic type with good physical fitness, and b) a neuroendocrine type with possible disturbances in the pituitary-adrenal system.

Thacker (1940a) performed cold pressor tests (Hines & Brown 1936) on 55 hypotensive 96 hypertensive and 128 normotensive students. The following results were obtained. Hypertensive and hypotensive classes reached the maximum increase in the systolic and diastolic blood pressures more slowly than the normotensives the same slow reaction was found on returning to the basal level. The systolic rise was 10 mm. Hg in normals, 11 mm Hg in hypotensives and 18 mm. Hg in hypertensive subjects. The corresponding rise in the diastolic blood pressure was 14 mm. Hg 11 mm. Hg and 16 mm. Hg, respectively The same author (1940 b) studied also the response of the pulse rate and blood pressure to an exercise test consisting of raising the weight of the body from the floor onto a chair 18 1/2 inches high at a rate of 40 cycles per minute. No difference in the return of the pulse rate to the basal level after the exercise was observed between normal and hypotensive subjects. The average increase of the systolic blood pressure evoked by exercise was 37 mm. Hg in the normals and 23 mm. Hg in hypotensive subjects. The diastolic pressure decreased in controls by 12 mm. Hg and in hypotensive subjects by 13 mm. Hg

In 1952 and later in 1955 Pellegrini summarized the results obtained by him and members of his staff (Bobba 1952 Malamani 1952 Malamani & Capra 1952 Pellegrini Piotti & Baldrighi 1952 Martignoni, Piotti & Drovanti 1953 Bobba & Baldrighi 1954 Baldrighi 1955 a b c, d Cei, Malamani & Tronconi 1955 Malamani 1955 a b Malamani & Mura 1955) The subjects studied by these authors were defined as patients exhibiting persistently low systolic and diastolic pressures without a reduction in the pulse pressure. Using the heart catheterization technique, the pressures of the right heart and pulmonary artery were found to be within normal limits or occasionally elevated. The left ventricular pressure was correlative to the low systemic arterial pressure In the experience of the authors the direct Fick method for determination

of the cardiac output gave too high values throughout. The cardiac output obtained by Fick was therefore reduced by a correction factor derived from the values obtained by the physical method of Böger & Wezler. The total blood flow was found to be within normal limits. The mean for the 17 tabulated patients was a cardiac index of 2.0 l. min. This figure for the normals was 2.64 l. min. Also the stroke volumes were within normal limits. The total blood volume, determined by a dye method, was 85.2 ccm. kg., compared with a mean value of 82.3 ccm. kg. of the controls. Nothing abnormal could be found in the circulation times in the lesser and greater circuits. The heart volume was determined according to Jonsell (1939) and the following values were obtained: Hypotensive subjects, mean 640 ccm., range 510—820 ccm. control subjects, mean of 740 ccm. range 625—850 ccm. When the heart volume was divided by the body weight no significant difference was found. Finally the pulse rate at rest was quite normal and no tendency towards a vagotonic pulse was observed.

The syndrome of arterial hypotension, a term used by Pellegrini, was defined as a state of decreased tone of the smaller arteries and arterioles, resulting in a reduced peripheral resistance.

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OBJECT OF PRESENT INVESTIGATION

Because of its pathological significance, hypertension has been extensively studied. As a result there is but little controversy concerning hemodynamic adjustment in hypertensive cardiovascular disease. Correspondingly the normal blood pressure and the inherent hemodynamic indices have been established as a result of primary interest and as a reference point to abnormal variations. On the other hand, no clearcut conclusions can be drawn on the circulation in essential hypotension.

In order to yield additional information concerning essential hypotension, the present study was carried out with an attempt to avoid some of the discrepancies evident from the survey The blood pressure figure was the only indication used for selecting subjects for study Special attention was also paid to persistence of the low blood pressure, a feature mostly ignored in earlier studies.

Among the various facets of circulation, it was the purpose to emphasize especially the following details: What is the basic circulatory parameter responsible for the low arterial pressure What is the level of cardiac pressure work? What is the circulatory response to exercise

regulation of the peripheral circulation.

The heterogeneity of the examined population is well illustrated in many surveys of the subject (Durant 1942, Bernhardt 1943 Laberke 1952 Schaefer 1954 Hueber & Thaler 1955 Pierach & Heynemann 1959)

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MATERIAL

To obtain a cross section of the hypotensive population the following arrangement was arrived at. About 10000 records in the sick funds of large industrial plants were examined. All male subjects with a systolic pressure of 110 or less were requested to arrive for a blood pressure measurement by the author. A few subjects were also obtained as volunteers from the Finnish Students Health Service and from the Institute of Occupational Health, Helsinki. An age range of about from 20 to 40 years was chosen because of the steadiness of the blood pressure through these years, allowing the application of the same blood pressure figure as a limit for hypotension. The control material was comprised of normotensive men in the same age range and from the same sources.

The blood pressure of the volunteers was measured in the late afternoon or in the evening after half an hour's rest in the supine position. The blood pressure was recorded with an accuracy of 2 mm. Hg with a cuff containing a bag 14 cm. wide and 33 cm. long. The first appearance of the sounds was taken to indicate the systolic value. For the diastolic pressure both the point of abrupt diminution of sounds and the point of their final disappearance was noted. In examining the hypotensive subjects, all cases with a systolic pressure over 110 were rejected from further study whereas all others passed to the first stage of the investigation proper including a checking of their general health. In addition to a clinical examination attention was paid to hematological data, especially in the light of the known effects of anemic states on circulation (e.g. Bäckman 1961). The acceptable lower limits of the hemoglobin content and erythrocyte count, and the upper limit of the sedimentation rate were 11 g./100 ml., 4 mill./ml. and 10 mm./1 h., respectively.

The total series of hypotensive subjects included 23 men. In five of them, low blood pressure values were observed during two months, in all the others from one to five years, mean 2.6 years. The mean age was 25 years, with a range of 19—37 years. Fourteen of the subjects were sedentary workers and nine laborers. Seventeen subjects had never participated in active sports, whereas six had years ago had athletic achievements and later on continued it only as a hobby.

The control material consisted of 21 normotensive men of a mean age of 23 /s years and range from 19—35 years. Sixteen were sedentary workers and five laborers. Seven of the subjects had earlier taken part in athletics but did so only occasionally at the present time.

Smokers were equally present in both groups.

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The dye dilution technique was employed for determining the cardiac output, using Evans blue (T 1824) [1] as indicator. The dye was rapidly injected into an antecubital vein from syringes accurately calibrated with two stoppers on the piston. Immediately after the injection the arm of the subject was raised to about 70° from the horizontal. The curve was continuously recorded from the heated ear pinna with the aid of an ear oximeter [2] based on the design of Wood & Geraci (1949). The lamp of the ear attachment was kept on for 15 minutes before the dye was injected. The subject breathed oxygen for 5 minutes before and during the whole procedure. By these means a stable baseline was obtained in all cases. The calibration sample was taken from the Cournand needle approximately 3 minutes after the dye injection and the corresponding time was marked on the curve. The dye from the plasma sample was extracted according to Jarnum (1959) and the analysis performed in a Beckman B spectrophotometer at a wave-length of 620 mμ (10 mm glass cuvettes). Double hematocrit readings were obtained by centrifuging arterial blood samples in capillary tubes for 30 minutes at a speed of 3000 r.p.m. The radius of the centrifuge was 10 cm. No correction was made for trapped plasma.

With the aid of the dye concentration of the calibration sample the dilution curve was converted to a concentration curve and plotted on semilogarithmic paper. The curve was analyzed with integrals directly from the semilogarithmic paper (Lilienfield & Kovach 1956) with special attention to the straightness of the downslope of the curve. The principle of the method of Lilienfield & Kovach is as follows: Semilogarithmically plotted dilution curves can be divided into the rapidly rising upslope + the curved portion of the downslope and, on the other hand, to the linear portion of the downslope. The total area of the curve is the sum of the area from the appearance time to the beginning of the linearity of the downslope and the area from that point to infinite time. The area of the first part of the curve is obtained by summation and the second area is obtained by graphical integration. The plasma flow is calculated by dividing the amount of injected indicator by the total integral. The cardiac output was obtained by multiplying the plasma flow with the hematocrit correction of $\frac{100}{100 - H}$. The mean circulation time was derived from the same semilogarithmic plotting according to Lilienfield & Kovach.

The blood sample for determining the blood volume was taken from the Cournand needle 10 minutes after the dye injection. The dye was extracted and the analysis performed exactly as in the calibration sample. The plasma volume was calculated by comparing the unknown with values obtained from known dye concentrations run

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[1] Vial No 12100, manufactured by Georg T Gurr London.

[2] Cambridge Instrument Company London.

METHODS AND PROCEDURE

Methods

With a few exceptions the investigation was performed in two stages. The first stage included a thorough clinical examination, and taking of the blood samples for determination of the sedimentation rate hemoglobin concentration and cell counts, and of samples for urine analysis. These samples were treated by routine techniics. Body measurements were made according to the recommendations of the Conference on the Role of Body Measurements in the Evaluation of Human Nutrition (Brožek 1956) also used earlier at this laboratory (Frick & Halonen 1961) The following measurements were made Weight, height, bi-acromial diameter bi-cristal diameter and upper arm circumference The body surface area (BSA) in square meters was obtained from nomograms based on Du Bois formula (1916) Chest x rays were taken in the upright position without correlation to the electrocardiogram. The heart volume was determined according to Jonsell (1939)

The second stage consisted of the hemodynamic measurements proper The electrocardiogram including standard and unipolar limb leads and precordial leads, was registered by a four *channel photoelectric device*[1] or exceptionally by a two channel direct writing Elema apparatus. The orthostatic ECG-test, as used by Holmgren *et al.* (1957 a b) was made in cases with a history suggestive of orhostatic reactions. The blood pressure was recorded from the brachial artery via an indwelling Cournand needle by means of a Statham P 23 AA pressure transducer and an *Atlas pressure device* The baseline for the pressure measurements was the middle of the chest at the level of the second costal cartilage (Roy Gadboys & Dow 1957) The manometer was repeatedly calibrated against mercury and the pressure did not deviate more than a maximum of 2 mm. Hg at the 200 mm. Hg level. The tracings were visualized with the aid of a cathode-ray oscilloscope and thus a damping could be observed and corrected. The mean pressure at rest was obtained by electrical integration. The mean pressure of the continuously recorded exercise pressure curves was calculated by planimetry

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1 Atlas Universal four channel writer Atlas Werk Bremen.

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through the same extraction procedure. The total blood volume was calculated with the aid of the hematocrit value in the usual manner

Jarnum's extraction procedure is as follows 4 ml. of heparinized plasma is pipetted into a glass-stoppered test tube and 5 ml. of 10 per cent sodium lauryl sulfate, the detergent, and about 0.25 g of Whatman's ashless cellulose powder are added. The tube is well shaken and the content is quantitatively transferred to an Allen's extraction tube When the fluid has passed through the filter the broth is removed with 0.5 ml. of a mixture containing 9 volumes of methanol and 1 volume of 9 per cent sodium chloride. The paper is then acidified by adding about 1 ml. of 0 1 N hydrochloric acid. Elution is carried out in two steps, 5 minutes apart, with a mixture of acetone and 0.2 N hydrochloric acid in the ratio 1 1

The ergometer used in the present investigation was a slight modification[1] of the ergometer described by v Döbeln (1954) It consisted of a frame and pedalling mechanism similar to that of an ordinary bicycle, but the transmission was connected to the front wheel. For the back wheel was substituted a stable construction under the saddle. The load was imposed by a friction band in a special furrow of the front wheel. The friction band was connected to a sinus balance for determining the torsional moment. The rate was controlled with a speedometer The ergometer was easily fixed with an extra device to an Elema-Schönander catheterization table for exercise studies in the supine position.

The physical working capacity was determined according to the outlines given by Sjöstrand (1947) and Wahlund (1948) and as used extensively since then (e g Sjöstrand 1951, Holmgren *et al.* 1957 a, b) The work test was begun with 300 kg.m./min. and the load was increased stepwise until the subject reached a pulse frequency of 170 beats/min. or more The pulse rate was counted every third and sixth minute of the load. Each load lasted 6 minutes. The physical working capacity was defined as the work in kg.m./min. performed by the subject at a pulse rate of 170 beats/min. and without a change of more than 10 beats/min. from the third to the sixth minute In case the pulse rate was higher at a given load the physical working capacity was obtained by graphical interpolation. All the subjects were able to reach a pulse frequency of 170 beats min. Consequently no extrapolation was necessary

The following formulas were used in calculating the various parameters

Central blood volume (CBV) = $\frac{MCT \times CO}{60}$ where MCT = mean circulation time and CO = cardiac output in liters.

Peripheral resistance (PR) = $\frac{BAP_{mean} \times 1332}{CO \text{ in ml./sec.}}$ dyn.sec.cm.$^{-5}$ where

BAP_{mean} = mean brachial arterial

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[1] Constructed at the Institute of Occupational Health, Helsinki.

pressure in mm. Hg, CO = cardiac output, and 1332 = conversion coefficient.

Left ventricular work index (LVWI)
$= \frac{CI \times BAP_{mean} \times 13.6}{1000}$ kg m./min./m² BSA, where CI = cardiac index = cardiac output in l. min./m² BSA, BAP_{mean} = mean brachial arterial pressure in mm. Hg, and 13.6 = the specific gravity of mercury.

Left ventricular stroke work index (LVSWI) $= \frac{SI \times BAP_{mean} \times 13.6}{1000}$ gm.m. m² BSA, where SI = stroke index = stroke volume in ml m² BSA, BAP_{mean} = mean brachial arterial pressure in mm. Hg, and 13.6 = the specific gravity of mercury. Since the pulmonary capillary venous pressure was not measured, no estimated value for it was subtracted from the mean brachial arterial pressure.

Estimates of central elasticity were calculated from the brachial arterial pressure tracings using the fractional rate of fall in pressure during diastole according to Landowne, Brandfonbrener & Shock (1955).

The electrocardiographic tracings were analysed with conventional criteria (Lepeschkin 1951, Sodi-Pallares & Calder 1956, Goldman 1958). The P—Q time was rate corrected according to v. der Weth (1939) and the Q—T interval using a nomogram given by Goldman (1958). In interpreting S—T segments a rise of 2 mv. or more in the precordial leads was held to be an elevation.

The parameters relating to square meter of body surface were obtained by dividing the crude values by BSA.

Current statistical methods have been used for the calculation of the arithmetical mean (M.), standard deviation (S.D.) and standard error of the mean (S.E.) and for studies of relationships with regression analyses (e.g., Cramér 1946). A factor analysis was performed according to the outlines given by Vahervuo & Ahmavaara (1958).

Procedure

For practical reasons the investigation was performed in the afternoon or in the evening, after at least a 6-hour fast. Smoking was avoided at least hours before the study. When the subjects arrived at the laboratory they were allowed to rest for about half an hour, after which the ECG was taken in the usual way and in some cases with a history suggestive of orthostatic reactions, after standing for 5 minutes. Then the tissue around the left brachial artery in the cubital fossa was liberally infiltrated with Xylocain[R] and an indwelling Cournand needle was inserted. The blood pressure was recorded with a sphygmomanometer before and after the procedure and care was taken to avoid even a minimal discomfort and pain which might have caused alterations in the blood pressure. The intra-arterial blood pressure was recorded after approximately an hour's bed rest. The ear pinna was then flushed with Finalgon[R] and the ear attachment

through the same extraction procedure. The total blood volume was calculated with the aid of the hematocrit value in the usual manner

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DISCUSSION OF METHODS AND PROCEDURE

Heart volume. The x ray measurement of the heart was introduced by Moritz (1902) who also was the first to determine it as a volume from three dimensions (1905). About ten years later Rohrer (1916) developed a formula based on determination of the volume of a geometric figure: $H_v = k \times F \times l_{max}$, F being the orthodiagraphically determined cardiac area, l_{max} the largest horizontal depth of the heart in the lateral picture, and k a constant. Kahlstorf (1932) re-examined the method using a series of 120 persons and reached the same formula. Based on extensive studies, Jonsell (1939) suggested that the frontal area may be calculated from the formula for an ellipse and arrived at $V = k \times l \times b \times d$, where V is the heart volume in cubic centimeters, l and b the axes of the ellipse, and d the maximum depth of the heart in the line of projection. K is a constant dependent on the geometrical calculation and magnification. Jonsell's method offers a more practical and objective means of measuring heart volume than the planimetric recording of Rohrer & Kahlstorf and it has been used extensively (e.g., Lind 1950, Linderholm & Strandell 1958). The method has been in use at the Wihuri Research Institute since 1947 and because of its evident advantages and some personal experience (Frick & Halonen 1960, Frick, Konttinen & Sarajas 1961) it was applied to the present investigation.

The length diameter was measured from the junction of the aorta and the right auricle to the apex of the heart. The broad diameter was measured from the junction of the diaphragmatic and right borders to the junction of the pulmonary conus and the left ventricle. Whenever possible the length and broad diameters were at right angles. The depth of the heart was measured from the most dorsal point of the heart shadow to the anterior outline of the heart at right angles to the vertebral column. The focus was at a distance of 1.75 meters. A value of 0.42 was given to the constant k.

Since the report of Dietlen (1909) it has been known that the heart volume is smaller in the erect position than in recumbency (Bjure & Laurell 1927, Moritz 1934, Nylin 1934, Larsson & Kjellberg 1948, Kjellberg 1952,

placed in position. After the lamp had been on for 15 minutes and the subject had breathed oxygen for 5 minutes, the first dye curve was made. After taking the calibration sample and the sample for blood volume determinations, the subject, still in supine position began to pedal the ergometer fixed to the catheterization table A uniform load of 400 kg.m./min. was used for all the subjects. The blood pressure was continuously recorded via the Cournand needle. The pulse rate was controlled by auscultation at the fifth and sixth minutes of exercise. When a steady state was reached, with two exceptions at the sixth minute the second dye injection was given and the curve was recorded. A blood sample was taken for the exercise hematocrit. The subject was then allowed to rest for half an hour after which the physical working capacity was determined in a sitting position

DISCUSSION OF METHODS AND PROCEDURE

Heart volume. The x ray measurement of the heart was introduced by Moritz (1902) who also was the first to determine it as a volume from three dimensions (1905). About ten years later Rohrer (1916) developed a formula based on determination of the volume of a geometric figure: $H = k \times F \times l_{ms}$. F being the orthodiagraphically determined cardiac area, l_{ms} the largest horizontal depth of the heart in the lateral picture, and k a constant. Kahlstorf (1932) re-examined the method using a series of 120 persons and reached the same formula. Based on extensive studies, Jonsell (1939) suggested that the frontal area may be calculated from the formula for an ellipse and arrived at $V = k \times l \times b \times d$, where V is the heart volume in cubic centimeters, l and b the axes of the ellipse, and d the maximum depth of the heart in the line of projection. K is a constant dependent on the geometrical calculation and magnification. Jonsell's method offers a more practical and objective means of measuring heart volume than the planimetric recording of Rohrer & Kahlstorf and it has been used extensively (e.g., Lind 1950, Linderholm & Strandell 1958). The method has been in use at the Wihuri Research Institute since 1947 and because of its evident advantages and some personal experience (Frick & Halonen 1960, Frick, Konttinen & Sarajas 1961) it was applied to the present investigation.

The length diameter was measured from the junction of the aorta and the right auricle to the apex of the heart. The broad diameter was measured from the junction of the diaphragmatic and right borders to the junction of the pulmonary conus and the left ventricle. Whenever possible the length and broad diameters were at right angles. The depth of the heart was measured from the most dorsal point of the heart shadow to the anterior outline of the heart at right angles to the vertebral column. The focus was at a distance of 1.75 meters. A value of 0.42 was given to the constant k.

Since the report of Dietlen (1909) it has been known that the heart volume is smaller in the erect position than in recumbency (Bjure & Laurell 1927, Moritz 1934, Nylin 1934, Larsson & Kjellberg 1948, Kjellberg 1952,

Holmgren & Ovenfors 1960) The alterations in heart volume are probably related to variations of ventricular filling pressure caused by changes in the central blood volume due to orthostatic blood distribution (Kjellberg, Rudhe & Sjöstrand 1949 Sjöstrand 1952 1953) The *reduction of* the heart volume by change from *recumbency to the erect position* has been observed to be inversely related to response of pulse frequency (Larsson & Kjellberg 1948 Kjellberg 1952) The heart volume is also reduced by drug induced tachycardia in the erect position but not in the supine position (Kjellberg 1952) That the methods used in calculating the heart volume may have some influence on the comparison of heart size in recumbency and in the prone position have been shown by Linderholm & Strandell (1958) Parallel measurements by the Larsson—Kjellberg and Jonsell methods showed a significant reduction of the heart volume in the erect position but the reduction was less with Jonsell s method. With both methods it was observed that the orthostatic pulse response had no significant effect on the relationship between the heart volumes in supine and erect positions. *Neither did the group of* vasoregulatory asthenia (Holmgren *et al.* 1957 a, b) with typical orthostatic pulse reactions differ from the control cases.

In the present investigation the heart volumes were measured from roentgenograms taken in erect position in connection with the routine clinical heart examination. This arrangement was considered accurate enough for comparison of the actual values between the different groups, although less accurate *for use in comparing the* heart volume with other hemodynamic indices *obtained in the supine* position.

Blood volume. *Since* Dawson, Evans & Whipple (1920) observed that the blue dye (T 1824) was more accurate in colorimetric readings than the great number of vital red dyes they studied, and after adaptation of this dye Evans blue by Gregersen, Gibson & Stead (1935) to plasma volume determinations, it has been widely used for determining the blood volume. The binding of the dye with albumin, its elimination rate tissue diffusion, and reliability in determining the blood volume as compared with other methods has been extensively studied and reviewed by numerous authors (e.g Reeve 1948 Barnes, Loutit & Reeve 1948 a b v Porat 1951 Ravdin, Walker & Rhoads 1953 Freinkel Schreiner & Athens 1953 Inkley Krieger & Brooks 1953 Sear Allen & Gregersen 1953 Frank & Gray 1953 Gregersen & Rawson 1959 Reeve 1960 Reeve, Allen & Roberts 1960 Remington & Baker 1961) Summing up it may be concluded that the dye is bound quickly and completely with albumin and eliminated slowly because of the strength of the bond between dye and albumin and further that comparisons with other methods for determination of plasma volume e.g., iodinated human serum albumin and radioactive chromium bound to plasma protein have shown close agreement,

whereas methods determining the red cell volume, e.g. radioactive iron, Cr^{51} radioactive phosphorus, agglutinated red cells, and carbon monoxide are usually considered to give lower blood volume values than those obtained with Evans blue.

Two methods of calculating the plasma volume with the aid of Evans blue are in general use. One consists of taking several consecutive samples at certain time intervals from injection, and extrapolation, either linear or semilogarithmic, to zero time. Another possibility is to calculate it with the aid of a single sample taken a sufficient length of time after the injection. The former management usually gives smaller plasma volumes because of escape of the plasma from the vascular system at a rate of about 1 ml. min., (v. Porat 1951).

In the present investigation the plasma volume and the blood volume determined with the aid of the hematocrit were calculated on the basis of a single sample taken 10 minutes after the dye injection. This course of action was selected to avoid an undesirable delay in the whole investigation procedure in which the first dye injection for determining the cardiac output and the blood volume was followed by another dye injection during the subsequent exercise period.

The direct methods of measuring the dye concentration of a plasma sample are not free from the inconvenience caused by lipemia, turbidity and hemolysis of the plasma. To avoid these several extraction procedures have been developed (e.g., Allen & Orahovats 1948, Allen 1951, 1953, Bedwell, Patterson & Swale 1955, Campbell, Frohman & Reeve 1958, Constable 1958, Tornberg 1958). Of them, the methods of Allen & Orahovats and of Allen seem to be the most widely used. Jarnum's extraction procedure (1959) used in the present investigation possesses certain advantages over the earlier ones. The fixed amount of paper eliminates the variation in the recovery of dye caused by uneven amounts of paper. Futher an acidified elution fluid is used, which increases the recovery of the dye. The method is rapid and fairly accurate. Methodical error counted from 23 paired determinations was 38 ml. per 1000 ml. of blood.

Cardiac output. The present study was begun by using a Cambridge cuvette oximeter and employing a vacuum to flow and an integral sample calibration (Theilen *et al.* 1955). However, very soon it became apparent that hypotensive subjects especially were sensitive to the bloodiness of the method and exhibited frequent vasovagal reactions that interfered with accurate pulse rate and stroke volume measurements. Hence all the results obtained up to that point were disregarded and the investigation was resumed using the ear oximeter.

This management was found to be well adapted to the purposes of the present investigation, in which it was deemed essential to reduce to a minimum the discomfort and apprehension caused by the procedure. This tendency, of course, is incorporated in any investigation procedure, but in

view of the known effect of apprehension on blood pressure changing a hypotensive subject to a normotensive one this special solicitude for the subject is justified.

The ear oximeter technique has not been widely used in quantitative determinations of the cardiac output (Beard & Wood 1951 Milnor *et al.* 1953 Gilmore *et al.* 1954 Bollinger 1955 Korner & Shillingford 1955 Kaufmann & Hegglin 1956 Kaufmann 1957 Sekelj Jegier & Johnson 1958 Hancock 1959 Taylor & Shillingford 1959 Denzler Rigotti 1961, McGregor Sekelj & Adam 1961) The main criticism to which this method has been subjected is doubt concerning the reliability of the transilluminated ear as a recording site of the instantaneous arterial dye concentration and concerning the validity of the (end) tail sampling calibration technique (cf Dow 1956) Quite recently McGregor *et al.* (1961) demonstrated that the capillary bed of the ear is perfused homogeneously enough for the purpose of recording a dye curve This conclusion was drawn from simultaneous dye curves recorded with a cuvette oximeter and an ear oximeter constructed to make tail sampling unnecessary (Sekelj & McGregor 1961) The validity of the calibration sample taken in the tail portion of the curve depends on the degree of vasodilatation and on the stability of the baseline depending on the constancy of the arterial oxygen content and instrumentation. Fluctuations in the former can be reduced to a minimum with the aid of oxygen breathing during the whole procedure. The stability of the instrument can be improved by using a low sensitivity and larger doses of dye. Current outlines of ear oximeter technique are described in detail by Gabe & Shillingford (1961)

The validity of the ear oximeter dilution curves is demonstrated by comparisons with Fick outputs (Gilmore et *al.* 1954 Korner & Shillingford 1955 Hancock 1959 Taylor & Shillingford 1959) with cuvette oximeter curves (Beard & Wood 1951, McGregor Sekelj & Adam 1961) and with outputs obtained with the multiple sampling method (Gilmore *et al.* 1954) These have in general shown fairly good correlation.

To test the earpiece and curve recorder used in the present study ten simultaneous dye dilution curves were made with the ear oximeter and with multiple arterial blood sampling (Hamilton *et al.* 1928 1932) (table 1) Differences from the values obtained with the multiple sampling technique varied from 0.2 to 19 8 per cent, mean 6 4 per cent. Checking of the reproducibility of the results obtained with the ear oximeter is presented in table 2. Repeated injections, approximately 10 minutes apart, gave almost the same mean value for cardiac output on both occasions, i.e., 6.61 l. min., and 6.62 l./min., respectively The mean difference between the outputs obtained from successive dye dilution curves was 7.3 per cent. Although the dye amount used in the second injection was exactly the same as that first injected, no damped response was

Case number	Hamilton dye	Ear oximeter	Difference	Difference percentage
1	4.48	4.05	−0.43	−9.6
2	6.19	6.39	+0.20	+3.2
3	5.73	5.83	+0.09	+1.6
4	7.05	7.42	+0.37	+5.2
5	5.89	6.01	+0.12	+2.0
6	10.13	8.52	−1.61	−15.9
7	8.02	8.30	+0.28	+3.5
8	7.32	8.77	+1.45	+19.8
9	8.53	8.63	+0.02	+0.2
10	8.18	8.42	+0.24	+2.9
Mean	7.25	7.33		
S.D.	1.77	1.71		
S.E.	0.54	0.54		

Table 1. Comparative cardiac outputs (L/min.) obtained with multiple sampling technique and ear oximeter

Case number	First injection	Second injection	Difference	Difference percentage
1	6.01	6.71	+0.70	+11.6
2	4.40	4.80	+0.40	+9.1
3	6.55	6.88	+0.33	+5.0
4	7.72	8.47	+0.75	+9.7
5	5.05	5.41	+0.36	+7.1
6	6.38	6.13	−0.25	−3.9
7	8.77	7.61	−1.16	−13.0
8	8.52	8.30	−0.22	−2.6
9	6.44	6.30	−0.14	−2.2
10	4.86	4.86	0	0
11	5.09	5.58	+0.49	+9.6
12	9.55	8.42	−1.13	−14.2
Mean	6.61	6.62		
S.D.	1.70	1.35		
S.E.	0.45	0.38		

Table 2. Cardiac outputs (l/min.) obtained with ear oximeter after two successive dye injections at interval of 10 minutes

observed. Figure 1 demonstrates the similarity of two successive curves.

The curves obtained by peripheral dye injection are usually not so steep as those obtained by a central injection. They tend to be somewhat prolonged and the recirculation hump less clearly discernible (fig. 1). When the area of these curves is estimated as usual by planimetry after linear replotting, the long, narrow area beneath the 0.1 line on the semilogarithmic chart is a certain source of planimetric error. The graphical method used in the pres-

view of the known effect of apprehension on blood pressure changing a hypotensive subject to a normotensive one, this special solicitude for the subject is justified.

The ear oximeter technique has not been widely used in quantitative determinations of the cardiac output (Beard & Wood 1951, Milnor *et al.* 1953 Gilmore *et al.* 1954 Bollinger 1955 Korner & Shillingford 1955 Kaufmann & Hegglin 1956 Kaufmann 1957 Sekelj Jegier & Johnson 1958 Hancock 1959 Taylor & Shillingford 1959 Denzler-Rigotti 1961, McGregor Sekelj & Adam 1961) The main criticism to which this method has been subjected is doubt concerning the reliability of the transilluminated ear as a recording site of the instantaneous arterial dye concentration and concerning the validity of the (end) tail sampling calibration technique (cf Dow 1956) Quite recently McGregor *et al.* (1961) demonstrated that the capillary bed of the ear is perfused homogeneously enough for the purpose of recording a dye curve This conclusion was drawn from simultaneous dye curves recorded with a cuvette oximeter and an ear oximeter constructed to make tail sampling unnecessary (Sekelj & McGregor 1961) The validity of the calibration sample taken in the tail portion of the curve depends on the degree of vasodilatation and on the stability of the baseline depending on the constancy of the arterial oxygen content and instrumentation Fluctuations in the former can be reduced to a minimum with the aid of oxygen breathing during the whole procedure The stability of the instrument can be improved by using a low sensitivity and larger doses of dye. Current outlines of ear oximeter technique are described in detail by Gabe & Shillingford (1961)

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To test the earpiece and curve recorder used in the present study ten simultaneous dye dilution curves were made with the ear oximeter and with multiple arterial blood sampling (Hamilton *et al.* 1928, 1932) (table 1) Differences from the values obtained with the multiple sampling technique varied from 0.2 to 19.8 per cent, mean 6.4 per cent. Checking of the reproducibility of the results obtained with the ear oximeter is presented in table 2. Repeated injections, approximately 10 minutes apart, gave almost the same mean value for cardiac output on both occasions, i.e., 6.61 l/min., and 6.62 l/min., respectively The mean difference between the outputs obtained from successive dye dilution curves was 7.3 per cent. Although the dye amount used in the second injection was exactly the same as that first injected no damped response was

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3	5.73	5.82	+0.09	+1.6
4	7.05	7.42	+0.37	+5.2
5	5.89	6.01	+0.12	+2.0
6	10.13	8.52	−1.61	−15.9
7	8.02	8.30	+0.28	+3.5
8	7.32	8.77	+1.45	+19.8
9	9.53	9.55	+0.02	+0.2
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8	8.52	8.30	−0.22	−2.6
9	6.44	6.30	−0.14	−2.2
10	4.86	4.86	0	0
11	5.08	5.48	+0.40	+7.9
12	9.35	8.02	−1.33	−14.2
Mean	6.61	6.82		
S.D.	1.70	1.55		
S.E.	0.49	0.38		

Table 2. Cardiac outputs (l./min.) obtained with ear oximeter after two successive dye injections at interval of 10 minutes

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that the oxygen uptake the A V oxygen difference and the cardiac output attained steady state already after the first minute of exercise in 15 of the subjects. Varnauskas (1955) made cardiac output determinations after 8 minutes of exercise in a series consisting of normal and hypertensive subjects. Recently Levy Tabakin & Hanson (1961) reported observations on the behavior of the cardiac output in normal subjects over prolonged periods of exercise, up to 20 minutes. A steady cardiac output was observed over a period of 5 to 10 minutes but wide variations occurred during the additional 10 to 15 minutes of exercise

Contrary to earlier studies, the exercising subjects were erect on a treadmill. Dye curves were recorded using a cuvette. The blood loss, calculated from the withdrawal speed and calibration samples, was about 150—200 ml.

In the present investigation the term «steady state means stabilized pulse rate and blood pressure and, with a very slight margin of error a stabilized cardiac output, since duplicate dye curves during exercise made at random on six subjects, showed a difference within the limits of methodical error

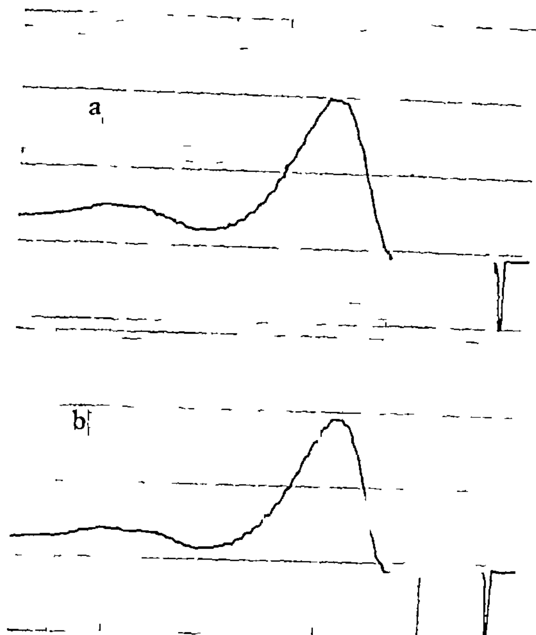


Fig. 1. Two successive dye curves. a) CO = 6.55 l./min., b) CO = 6.88 l./min.

ent investigation (Lillenfield & Kovach 1956) was found to be very suitable and accurate eliminating the aforementioned possibility of error.

Steady state. Exercise studies on normal subjects have shown slightly different time limits for attainment of the steady state. Dexter *et al.* (1951) made exercise tests of rather short duration, i.e., 2 to 3 minutes. The same type of short exercise periods were used by Hickam & Cargill (1948). The validity of these earlier studies was confirmed by Donald *et al.* (1955) in a very thorough study on 16 normal subjects. They found

a slightly broader shoulder girdle than the hypotensive subjects, the mean values of the bi-acromial diameters being 39.8 and 37.7 cm., respectively. The arm circumference of the hypotensive subjects was on the average 2 cm. shorter than that of the controls.

Electrocardiography. The absolute and heart rate corrected P—Q times and Q—T intervals in the two groups showed no definite differences. The mean values of the P—Q and P—Q_c of the controls were the same, 0.16 sec., and of the hypotensive subjects 0.16 and 0.17 sec. For the Q—T intervals the figures were 0.35 and 0.37 sec. for the controls and 0.37 and 0.37 sec. for the hypotensives. One control subject had a P—Q of 0.30 sec. (P—Q_c = 0.16) revealing first degree of a-v block. The tracing was otherwise normal and the subject in good physical health. An S—T elevation in two or more precordial tracings occurred in four control subjects and 12 hypotensives.

Partial right bundle branch block occurred in the tracings of four control subjects (19 per cent) and seven hypotensive subjects (30 per cent). The tracings were otherwise normal. Neither the history nor the clinical heart status in these cases pointed to heart disease. A vertical heart position was found in the tracings of three of the control cases (14 per cent) and six of the hypotensive subjects (26 per cent).

An orthostatic ECG test, made on one control and four hypotensive subjects with a history suggestive of orthostatic reactions showed a normal response.

Hematology. The mean values of the hematological data are collected in table 4. The differences between the groups were only slight and insignificant. The hemoglobin values of the control subjects ranged between 11.0 and 15.5 g./100 ml., with a mean value of 13.3 g./100 ml. The values of the hypotensive subjects were from 11.1 to 16.4 g./100 ml., average 13.9

		Hb (g./100 ml.)	Hct (vol.%)	PV (liters)	RCV (liters)	TBV (liters)	TBV (L/m².BSA)
Mean	C	13.3	41.8	3.22	2.31	5.53	2.92
	H	13.9	42.4	2.96	2.27	5.23	2.86
Range	C	11.0—15.5	36.8—47.2	2.01—4.47	1.59—3.57	3.82—8.04	2.17—3.84
	H	11.1—16.4	34.0—48.4	2.16—4.14	1.22—2.92	3.58—6.96	2.00—3.67
S.D.	C	1.15	2.98	0.596	0.421	0.965	0.423
	H	1.35	3.11	0.514	0.370	0.835	0.443
S.E.	C	0.25	0.63	0.130	0.092	0.211	0.092
	H	0.28	0.65	0.107	0.077	0.174	0.092
	P						

Table 4. Details of hematological data. C = control subjects, H = hypotensive subjects, Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume

RESULTS

I. General observations

The group data of the anthropometric measurements are given in table 3 and of the hematological findings in table 4. The individual values of these findings and of the electrocardiograms, heart volumes and physical working capacity are listed in tables 9—16 in the Appendix.

Anthropometry. The mean weight of the control subjects was 74.2 kg., with a range of 64.5—91.0 kg. (table 3). The corresponding figures for hypotensive subjects ranged between 52.0 and 84.5 with a mean value of 68.9 kg. No attempt was made to determine exactly the underweight of the subjects, but at least six of the hypotensive individuals were rather lean (cases 22, 27, 28, 34, 36 and 41), their weight passing below the lower limit of control subjects. The only statistically significant difference in anthropometry was, indeed found in the body weight ($P < 0.05$). All the anthropometric parameters given later in this paper showed but insignificant differences between controls and hypotensive subjects.

The height of the control subjects ranged between 163 and 187 cm. with a mean of 178.2 cm. The average height of the hypotensive subjects was 176.2 and the range 166—190 cm. The mean body surface area in square meters was 1.89 for controls and 1.82 for hypotensive subjects.

The mean values for the width of the pelvic girdle were almost identical in the two groups, i.e. 28.6 and 28.8 cm. whereas control subjects showed

Parameter	Control Subjects			Hypotensive Subjects		
	Mean	S.D	S.E.	Mean	S.D	S.E.
Weight (kg.)	74.2	7.52	1.64	68.9	8.30	1.73
Height (cm.)	178.2	6.32	1.38	176.2	6.23	1.30
Body surface area (m^2)	1.89	0.12	0.03	1.82	0.13	0.03
Bi acromial diameter (cm.)	39.8	1.71	0.37	37.7	2.54	0.53
Bi-cristal diameter (cm.)	28.6	2.03	0.44	28.8	1.86	0.39
Arm circumference (cm.)	29.6	2.03	0.44	27.9	2.17	0.45

Table 3. Anthropometric measurements

a slightly broader shoulder girdle than the hypotensive subjects, the mean values of the bi-acromial diameters being 39.8 and 37.7 cm., respectively. The arm circumference of the hypotensive subjects was on the average 2 cm. shorter than that of the controls.

Electrocardiography. The absolute and heart rate corrected P—Q times and Q—T intervals in the two groups showed no definite differences. The mean values of the P—Q and P—Q_c of the controls were the same, 0.16 sec., and of the hypotensive subjects 0.16 and 0.17 sec. For the Q—T intervals the figures were 0.35 and 0.37 sec. for the controls and 0.37 and 0.37 sec. for the hypotensives. One control subject had a P—Q of 0.30 sec. (P—Q_c = 0.16) revealing first degree of a-v block. The tracing was otherwise normal and the subject in good physical health. An S—T elevation in two or more precordial tracings occurred in four control subjects and 12 hypotensives.

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		Hb (g./100 ml.)	Hct (vol.%)	PV (liters)	RCV (liters)	TBV (liters)	TBV (L/m BSA)
Mean	C	13.3	41.8	3.22	2.31	5.53	2.92
	H	13.9	43.4	2.96	2.27	5.32	2.86
Range	C	11.0—15.5	36.6—47.2	2.01—4.47	1.79—3.57	3.82—8.04	2.17—3.84
	H	11.1—16.4	34.0—48.4	3.16—4.14	1.22—2.82	3.59—4.96	3.00—3.67
S.D.	C	1.15	2.86	0.596	0.431	0.965	0.420
	H	1.33	3.11	0.514	0.370	0.835	0.443
S.E.	C	0.25	0.63	0.130	0.092	0.211	0.092
	H	0.28	0.65	0.107	0.077	0.174	0.092
	P						

Table 4. Details of hematological data. C = control subjects, H = hypotensive subjects, Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume

g /100 ml Arterial hematocrit values of the controls ranged from 36.8 to 47.2 per cent by volume and those of the hypotensives from 34 0 to 48 4 per cent by volume The corresponding mean values were 41.8 and 43 4 per cent by volume respectively

The plasma volumes of the controls ranged between 2014 and 4470 ml with a mean volume of 3221 ml., and those of the hypotensives between 2159 and 4143 ml., with a mean of 2956. The mean red cell volume was almost the same in the two groups, i.e., 2309 ml. and 2275 ml with ranges of 1792—3568 and 1222—2820 ml. respectively The mean of the total blood volume of the control subjects was about 300 ml. greater than that of the hypotensives the values being 5530 ml. and 5231 ml. respectively The blood volume varied between 3817 and 8038 ml. for the controls and between 3594 and 6963 ml. for the hypotensive subjects. When the total blood volume was plotted against the body surface area in square meters, the mean values for the two groups were almost the same i.e 2921 and 2859 ml., respectively These values varied between 2169 and 3940 ml. in the control group and between 2002 and 3665 ml in the hypotensive group.

The mean total blood volume in ml./sq.m. in the control group was almost identical with the results obtained with Evans blue in healthy males by Gibson & Evans (1937) and by Kyllönen (1961) the difference being 10 ml. and 22 ml. respectively Davis (1942) found a mean value of 2897 ml. for healthy males, whereas von Porat (1951) reached the somewhat higher figure of 3104 ml.

Heart volumes varied between 578 and 999 cc. for control subjects and between 470 and 977 cc. for hypotensive subjects. The mean values were 784 (S.D = ± 126.9) and 712 (S.D = ± 128.6) cc. respectively Plotting against body surface area revealed mean figures of 415 (S.D = ± 58.6) and 390 (S.D = ± 59.9) cc., and ranges of 314—540 cc. and 288—498 cc., respectively The mean values, either absolute or relative did not differ significantly

Contrary to the accepted upper normal limits of heart volume (e.g Jönsson 1957) the lower normal limits have not, to the knowledge of the writer been established. If an arbritrary limit of 600 cc. is chosen, corresponding to a heart volume of approximately 300—340 cc./sq.m in a man of average build, one of the control cases and three of the hypotensive subjects (cases 10 24 26 and 36) had small hearts. These could also have been called drop hearts on account of the characteristic shape of the heart shadow

Physical working capacity (PWC) The control cases showed a PWC of 720—1200 kg.m./min. and the hypotensive cases 510—1332 kg.m./min. The respective mean values, 956 (S.D = ± 138.9) and 967 (S.D = ± 167.8) kg.m /min. differed insignificantly from each other According to the experience of Holmgren *et al.* (1957 b) the PWC is usually not below 900 kg.m./min. in healthy men 20—40 years of age In the present series five control cases had PWC values of

less than 900 kg.m./min. The lowest value i.e., 510 kg.m./min. was found in a very asthenic hypotensive individual (case 36) This value shows that the load of 400 kg.m./min. uniformly used for all the subjects in the hemodynamic exercise studies and selected before starting the study was suitable for and easy to reach by the subjects.

II. Hemodynamic parameters at rest

The group data of hemodynamic findings are given in tables 5 & 6 and the individual values in tables 15—18 in the Appendix.

Pulse rate, beats/min. The control cases showed a variation of 50—84, with a mean of 68. Hypotensive subjects had a mean value of 59 (P < 0.01) with a range of 48—75 (table 5) Four of the controls had pulse rates below 60 beats/min., whereas this was the case in 12 of the hypotensives (19 per cent, as compared to 52 per cent)

Blood pressures, mm. Hg. The systolic intra-arterial blood pressure of the control subjects varied between 117 and 172, with a mean of 136.6. The corresponding mean of the hypotensive group was 109.9 (P < 0.001) and the values varied between 90 and 143 (table 5)

The diastolic pressures varied between 63 and 111 in the control group and between 57 and 80 in the hypotensive group. The corresponding mean values were 81.3 and 68.8 (P < 0.001)

The mean pressure was in the range 81—125 in the controls and 67—85 in the hypotensives. The mean value

		PR (beats/min.)	BAP systolic	BAP diastolic	BAP mean	BA pulse pressure
Mean	C	68.0	136.6	81.3	100.2	55.2
	H	58.8	109.9	68.8	80.8	41.0
Range	C	50—84	117—172	63—111	81—125	36—72
	H	48—75	90—143	57—80	67—85	26—71
S.D.	C	8.8	13.1	13.1	12.5	9.67
	H	7.2	10.3	8.3	7.2	10.7
S.E.	C	1.9	2.85	2.67	2.73	2.18
	H	1.5	2.15	1.32	1.80	2.24
	t	2.06	7.84	3.90	5.77	4.87
	P	0.01	0.001	0.001	0.001	0.001

Table 5. Details of pulse rate and blood pressure. C = control subjects, H = hypotensive subjects, PR = pulse rate, BAP = brachial arterial pressure, BA = brachial artery The pressure values are intra-arterial and expressed in mm.Hg

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Range	C	50—84	117—172	63—111	81—125	36—72
	H	48—75	90—143	57—80	67—85	26—71
S.D.	C	8.8	13.1	13.1	12.5	9.97
	H	7.2	10.3	6.3	7.2	10.7
S.E.	C	1.9	2.85	2.67	2.73	2.18
	H	1.5	2.15	1.22	1.50	2.24
	t	2.96	7.84	3.90	3.77	4.81
	P	0.01	0.001	0.001	0.001	0.001

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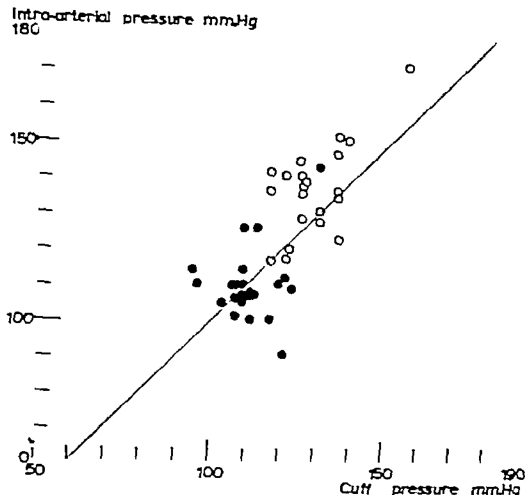


Fig 2. Relation between direct and indirect systolic blood pressures. O = control subjects, ● = hypotensive subjects

was 100.2 and 80.9 respectively (P < 0.001)

The pulse pressure of the control subjects varied between 36 and 72, mean 55.2. The hypotensive group showed a range of 26—71 and a mean of 41.0 (P < 0.001)

From the above data it may be concluded that the hypotensive subjects exhibited a decrease in both the systolic and the diastolic pressure, the systolic decrease being more marked and resulting in a decreased pulse pressure as well.

Since the pioneer studies of Wolf & von Bonsdorff (1931) and von Bonsdorff (1932) it has been repeatedly demonstrated that certain differences exist between intra-arterial and cuff pressure values (e.g., Hamilton, Woodbury & Harper 1936, Steele 1942, Van Bergen *et al.* 1954 Berliner *et al.* 1960 1961) and that the deviations are correlated to the arm circumference (Ragan & Bordley 1941) With regard to essential hypotension, in the study of which thin asthenic individuals form a great part of the series reported

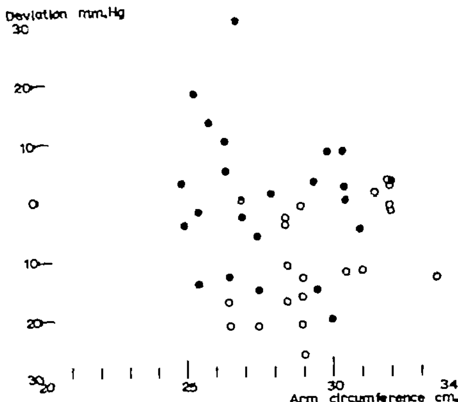


Fig. 3. Relation between arm circumference and deviation of indirect systolic pressures from intra-arterial values. O = control subjects, ● = hypotensive subjects

in the literature it is not entirely possible to disregard the assumption that a certain number of low blood pressure values are only cuff hypotension.

To elucidate this question with respect to th present blood pressure values, the direct brachial artery pressures and the indirect ones were compared. The cuff pressure was measured from the free arm immedi ately after registration of the direct tracing and was calculated for the arm with the indwelling needle by taking into consideration the bilateral cuff values before the needle insertion. Figure 2 illustrates the relation between the systolic pressures. In the control group the cuff method slightly underestimated the intra-arterial val ues ($P < 0.05$) whereas the difference was insignificant in the hypotensive group ($P > 0.80$) and in the total series ($P > 0.05$) No clearcut trend with regard to arm circumference was detectable (fig. 3) With regard to the diastolic pressure using of the point of muffling of the sounds caused

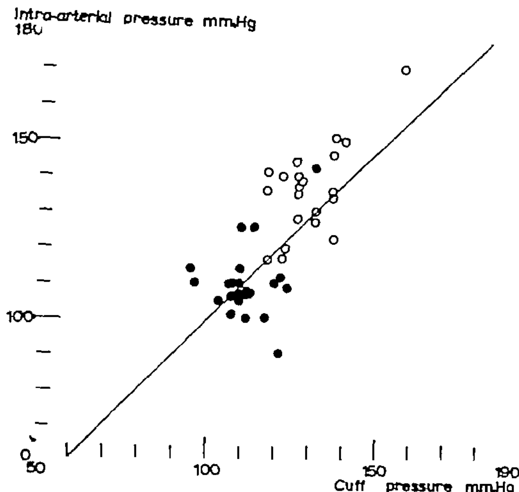


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		Mean	Range	S.D	S.E.	t	P
Circulation time (sec.)	C	10.7	8.0—13.4	1.25	0.27	3.18	0.01
	H	12.1	10.0—16.2	1.54	0.32		
Cardiac output (L/min.)	C	6.04	4.23—9.07	1.31	0.29	2.06	0.05
	H	5.11	3.69—6.94	0.85	0.19		
Cardiac index (L/min.)	C	3.21	2.29—4.82	0.71	0.16	2.11	0.05
	H	2.81	2.08—3.61	0.53	0.11		
Stroke volume (ml.)	C	92.3	57—130	20.3	4.44	0.90	
	H	86.0	54—110	14.6	3.05		
Stroke index (ml.)	C	48.9	31.3—68.8	11.1	2.40	0.16	
	H	48.5	31.5—60.0	2.49	0.52		
Mean circulation time (sec.)	C	20.8	14.5—28.1	3.8	0.8	2.23	0.05
	H	23.9	18.6—33.7	5.1	1.1		
Central blood volume (L)	C	2.08	1.22—3.17	0.48	0.10	0.51	
	H	2.01	1.14—2.74	0.42	0.09		
Central blood volume (L/m^2 BSA)	C	1.10	0.69—1.72	0.28	0.06	0.06	
	H	1.11	0.84—1.41	0.22	0.05		
Peripheral resistance (dyn.sec.cm^{-5})	C	1378	827—1978	314	68.6	0.67	
	H	1324	911—1798	288	60.2		
Left ventricular work index (kg.m./min./m^2 BSA)	C	4.4	2.5—6.5	1.1	0.2	4.71	0.001
	H	3.1	2.3—4.4	0.6	0.1		
Left ventricular stroke work index (gm.m./m. BSA)	C	67.0	42.6—103.8	16.8	3.67	8.06	0.001
	H	53.3	35.6—71.4	9.06	1.99		
Central elasticity (dyn./cm^2/cm^3)	C	602	234—1086	215	47	0.89	
	H	541	203—971	178	37		

Table 6. Details of circulation times, blood flows, pressure flow relationships, and central blood volumes t rest. C = control subjects, H = hypotensive subjects

time, appearance time) It varied between 8.0 and 13.4 in the control group and between 10.0 and 16.2 in *the hypotensive group*. The mean of the controls was 10.7 and that of the hypotensives 12.1 ($P < 0.01$) (table 6)

Cardiac output and cardiac index L/min. (table 6) As already could be assumed on the basis of the longer circulation time of the hypotensive subjects and an almost equal blood volume in both groups, the mean cardiac output of the hypotensives was smaller than that of the controls, the values being 5.11 and 6.04 ($P < 0.05$) respectively Naturally there was considerable overlapping, the values varying between 4.23 and 9.07 for the controls and from 3.69 to 6.94 in the hypotensive group. The corresponding cardiac index was in the range 2.29—4.82 in the control group and

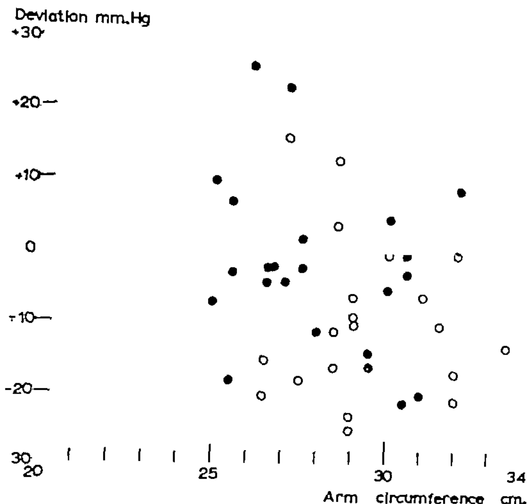


Fig. 4. Relation between arm circumference and deviation of indirect diastolic pressures from intra-arterial values. Cessation of sounds regarded as diastolic pressure. O = control subjects, ● = hypotensive subjects

insignificant overestimation of the intra-arterial values in the control group ($P > 0.30$) but more marked in the hypotensive group ($P < 0.05$) When the cessation of the sounds was regarded as the diastolic pressure the intra-arterial values were underestimated in the control group ($P < 0.02$) but were nearly equal in the hypotensive group ($P > 0.40$) In the total series the intra-arterial values were just at the midpoint between the two cuff values, differing from them insignificantly No evident trend was found on comparison of the arm circumference with the deviation of the diastolic pressure from the intra-arterial one (fig. 4)

Circulation time, sec. This was obtained from the dye dilution curves as the time interval from the moment of dye injection into the cubital vein to the *first deflection of the dye curve obtained from ear pinna* (arm to ear

downe & Shock 1955 Eisalo 1956) *Using the ear oximeter* Frick, Konttinen & Sarajas (1961) found a mean of 3.07 l/min. in healthy recruits. Thus the mean cardiac index of hypotensive subjects passes below the lower normal mean value reported in the literature. This is all the more evident since usually no differentiation has been made between normotension and hypotension in series of normal cases.

Stroke volume and stroke index, ml., (table 6) The existence of considerable variation in the stroke volume has been emphasized in the literature. Starting from the low values obtained with the acetylene technique, mean values as high as *118* with Hamilton dye have been reported (Freis *et al.* 1952) In the present study a mean stroke volume of 92.3 was found in the controls. Individual variations ranged from 57 to 130 The hypotensive group showed an average of 88, with a range of 54—110 The mean stroke index of the controls was 48.9 with a range of *31.3—68.8, and the* mean of the hypotensive group 48.5, with a range from 31.5 to 60.0 Differences between the groups were but slight and insignificant.

The stroke index values were almost the same as those reported for the corresponding age by Brandfonbrener Landown & Shock (1955) obtained by multiple arterial sampling.

In view of the significantly lower pulse pressures of the hypotensive subjects the almost identical mean stroke indexes are of some interest. Several formulas have been developed for the correlation between pulse pressure and stroke volume (cf. Handbook of Circulation 1959) including several constants and correction factors. The standard deviations of these correlations have usually been about 12 ml. To demonstrate the salient difference between the groups with regard to pulse pressure and *stroke volume, the data were correlated* as pulse pressure and stroke index, as done earlier by Remington *et al.* (1948) Figure 6 shows the scatter plot. It can be clearly seen that most of the hypotensive subjects have comparable stroke indexes but a lower pulse pressure than the control subjects.

The relation between the stroke volume and the central blood volume is shown in figure 7 The correlation was significant for the control group ($r = 0.85$ $P < 0.001$) for the hypotensive group ($r = 0.67$ $P < 0.001$) and for the total material ($r = 0.77$ $P < 0.001$)

The relation to the total blood volume showed a rather wide scatter (controls, $r = 0.14$ hypotensives, $r = 0.36$ total material, $r = 0.16$) which is illustrated in figure 8. The average stroke volume of both groups was 1.7 per cent of the blood volume

Mean circulation time, sec., (table 6) The mean of the controls was *20.8 and that of the hypotensive subjects* 23.9 ($P < 0.05$) The respective ranges were 14.5—28.1 and 18.6—35.7

Central blood volume, l. or l./m BSA, (table 6) The *absolute values*

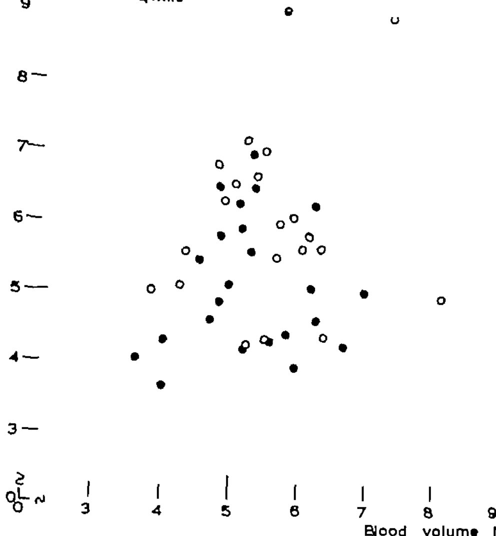


Fig. 5. Relation between cardiac output and blood volume. O = *control subjects*, ● = hypotensive subjects

2 08—3 61 in the hypotensive group. The respective mean values were 3.21 and 2.81 (P $<$ 0 05)

The ratio $\frac{\text{Blood volume}}{\textit{Cardiac output}}$ was *0.92* *for controls and 1 02 for hypotensive* subjects. The relation between cardiac output and blood volume is shown in figure 5 A wide scatter was found, r values being 0 12 for the controls, 0 07 for the hypotensive subjects, and 0 16 for the total material.

The mean cardiac index of normal males obtained by the dye method utilizing intermittent arterial sampling has varied between 2.9 and 4.1 l./min. (Hamilton *et al.* 1948 Ebert *et al.* 1949 Werkö *et al.* 1949· Doyle *et al.* 1951 Freis *et al.* 1952, Chapman & Fraser 1954 Brandfonbrener Lan

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Central blood volume, L or L/m² BSA, (*table 6*). The absolute values

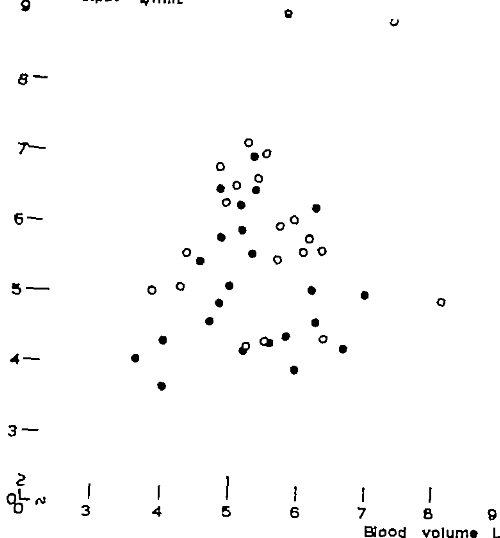


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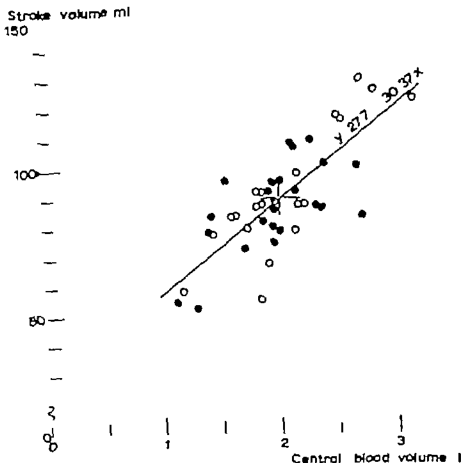


Fig. 7 Relation between stroke volume and central blood volume at rest.
O = control subjects, ● = hypotensive subjects

injection gave a mean value of 1.03 L/m² BSA for the central blood volume of subjects without cardio-vascular and pulmonary disease

Peripheral resistance, dynes sec. cm⁻⁵ varied between 827 and 1975 in control cases. The mean was 1376. Lagerlöf & Werkö (1949) reported a range of 882—1790 and a mean of 1190 in a series of 13 subjects. The range of variation in the 16 normal cases of Donald *et al.* (1955) was 591—1220 and the mean value was 876. In the two aforementioned studies the cardiac output was determined by direct Fick. Using dye dilution and cuff pressure, Eisalo (1956) found the mean peripheral resistance of 25 normal cases to be 1611, with a range of 1056—3419

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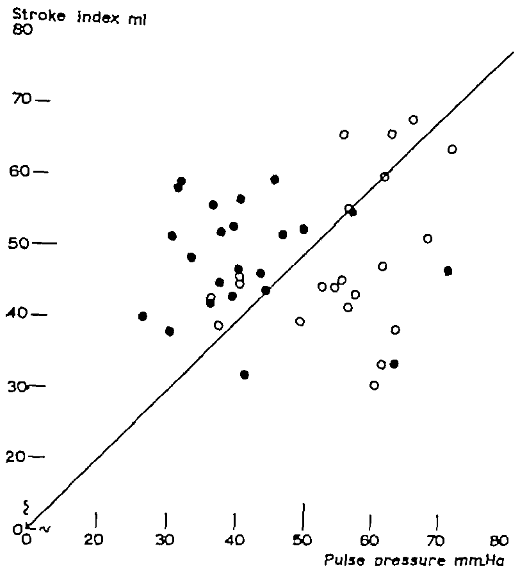


Fig. 6. Graph showing relation between stroke index and pulse pressure. O = control subjects, ● = hypotensive subjects

varied between 1.22 and 3.17 for the controls and between 1.14 and 2.74 for the hypotensive subjects. The corresponding ranges, when expressed per sq m. of body surface were from 0 69 to 1 72 in the control group and from 0 64 to 1.41 in the hypotensive group The mean of the crude values was 2 08 for the control group and 2.01 for the hypotensive subjects, exhibiting an insignificant difference. The same was true for the difference between the respective mean values calculated per sq.m body surface, i.e., 1.10 and 1.11.

Quite recently Denzler—Rigotti (1961) compared the validity of peripheral and central dye injections in measurements of the central blood volume. The curves were recorded with an ear oximeter A linear correlation was found. The peripheral

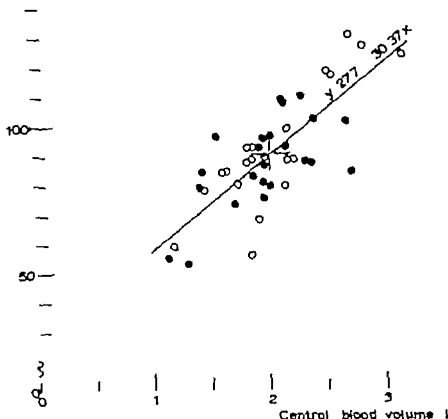


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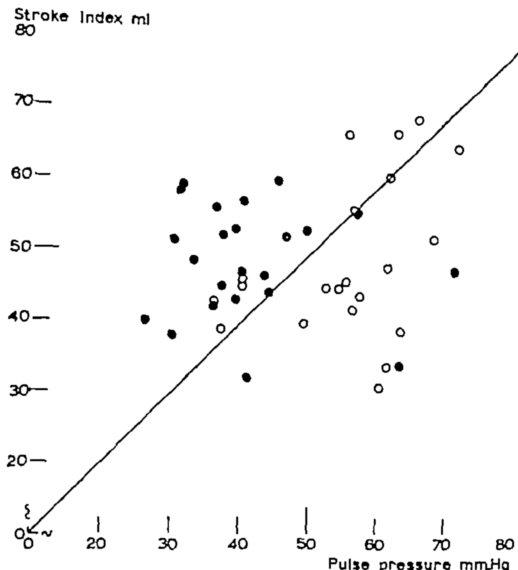


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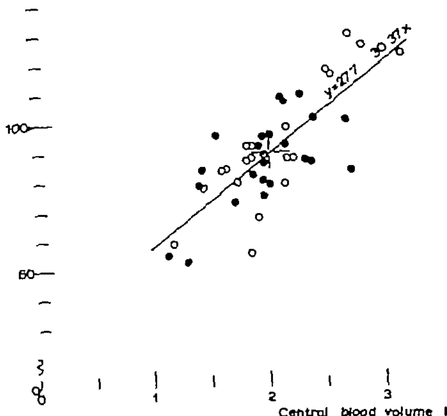


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The hypotensive cases showed variation from 911 to 1793 and a mean of

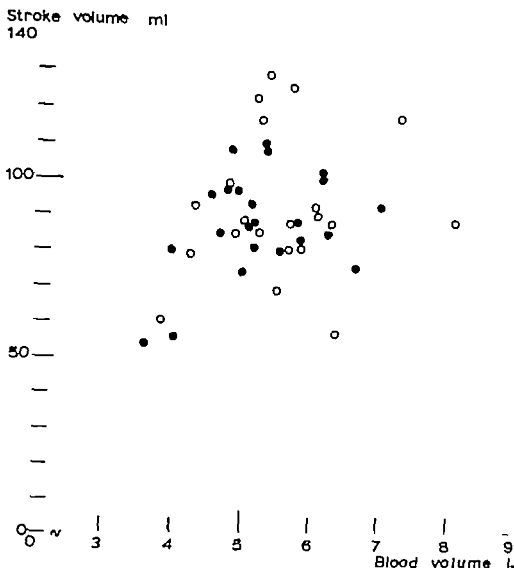


Fig 8. Relation between stroke volume and blood volume at rest. O = control subjects, ● = hypotensive subjects

1324 which differed insignificantly from the control mean.

Left ventricular work index, kg.m./min./m² BSA, showed a mean value of 4 4 in the control group, varying between 2.5 and 6 5 (table 6) This is somewhat lower than the mean of 5 0 of Lagerlöf & Werkö (1949) and of 5.1 of Donald *et al.* (1955) and about 25 per cent lower than the mean of 6.2 obtained by Varnauskas (1955) The mean cardiac indexes obtained by these authors were higher than the mean in the present series.

The mean of the hypotensive subjects was considerably lower than that of the controls, being 3 1 ($P < 0 001$) The range of variation was from 2.3 to 4 4 Thus the highest individual value equaled the control mean.

Left ventricular stroke work index, gm.m./m² BSA, (table 6) of the

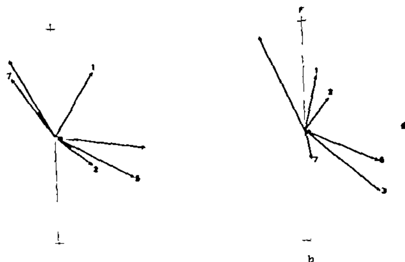


Fig. 9. Factorial loadings of hemodynamic parameters. = control group, b = hypotensive group, F_I = first factor F_{II} = second factor,1 = brachial arterial mean pressure, 2 = pulse pressure, 3 = cardiac index, 4 = peripheral resistance, 5 = central blood volume per sq.m. BSA, 6 = left ventricular stroke work index, 7 = heart volume per sq.m. BSA

control subjects varied between 42.6 and 103.8 the mean being 67.0 The mean of the hypotensive subjects was 53.3 ($P < 0.001$) with a range of 35.6—71.4. The corresponding mean values of normal cases in the series of Lagerlöf & Werkö (1949) and Varnauskas (1955) were 73 and 74 respectively

Central elasticity dynes/cm².cm³ varied between 236 and 1098 in the control group and between 203 and 971 in the hypotensive group. The control mean was 603, being about the same as the mean (593) for the corresponding age obtained by Landowne Brandfonbrener & Shock (1955)

The mean of the hypotensive group was 541 differing insignificantly from the control value (table 6) When the pulse pressure was estimated by taking elasticity into consideration, i.e., as a product of the stroke volume and the elasticity a mean value that was almost identical with the actual figure was obtained in the control group, the means being 54 and 55 mm. Hg, respectively In the hypotensive group the estimated pulse pressure was 46, as against 41 mm. Hg obtained from the pressure tracings ($P > 0.10$) When these elasticity corrected values of pulse pressure for the two groups were compared no statistical difference was evident ($P > 0.05$)

Factor analysis of the parameters revealed two factors, common to both groups, which determined the position of the parameters in the dynamic field (figure 9) The first factor was equal to the left heart pressure work and the second near the peripheral resistance. The other hemodynamic

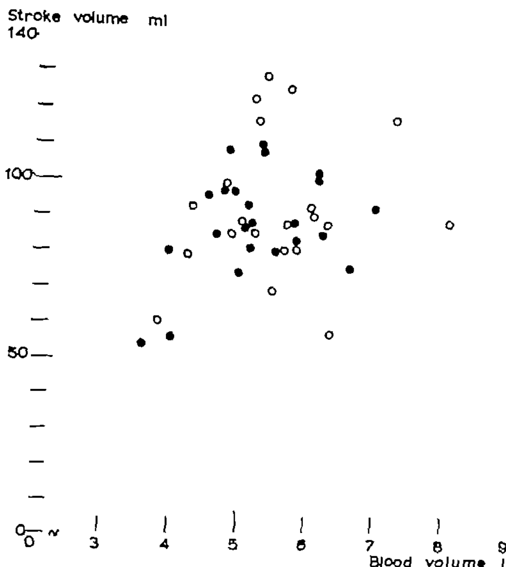


Fig 8. Relation between stroke volume and blood volume at rest. O = control subjects, ● = hypotensive subjects

1324, which differed insignificantly from the control mean.

Left ventricular work index, kg.m./min./m² BSA, showed a mean value of 4 4 in the control group varying between 2.5 and 6.5 (table 6) This is somewhat lower than the mean of 5 0 of *Lagerlöf & Werkö (1949)* and of 5 1 of Donald *et al* (1955) and *about 25 per cent lower than the* mean of 6.2 obtained by Varnauskas (1955) The mean cardiac indexes obtained by these authors were higher than the mean in the present series.

The mean of the hypotensive subjects was considerably lower than that of the controls, being 3 1 ($P < 0.001$) The range of variation was from 2.3 to 4.4 Thus the highest individual value equaled the control mean.

Left ventricular stroke work index, gm.m /m² BSA, (table 6) of the

III. Hemodynamic parameters during exercise

All the values were registered at a uniform load of 400 kg.m./min. between the sixth and seventh minute of exercise.

Group data are given in tables 7 & 8 and the individual values in tables 19 & 20 in the Appendix.

Pulse rate, beats/min. The mean increase was 46 in the control cases, with individual variations from 29 to 60. The mean percentile increment was 69 with a range of 39—108 per cent (table 7)

The hypotensive subjects exhibited a mean absolute increment of 48 and a percentile one of 81 per cent. The variations were from 30 to 66 and from 48 to 123 per cent, respectively. The discrepancy between the almost equal absolute increment of the pulse rate in both groups and the considerably different percentile increase is clarified in fig. 10. In this graph, lines are drawn from the pulse rates at rest to the physical working capacity values at the 170 pulse rate level. It can be seen that the percentile increments in the pulse rate at the 400 kg. m./min. level used in the exercise studies differ by 11 per cent on account of the lower starting level of the hypotensive subjects. The percentile pulse rate increments obtained from this composed graph are almost identical with the actual figures cited above obtained from the exercise hemodynamic studies. This proves the validity of the correlation between the pulse rate response to exercise and the physical working capacity

There was no significant difference between the groups with respect to either the change or the absolute values during exercise.

Blood pressures, mm.Hg, (table 7) Distinctly undamped pressure curves were obtained in 20 cases, including 8 controls and 12 hypotensive subjects.

	N	At rest		During exercise		Change		
		Mean	Range	Mean	Range	Mean	Range	Mean %
Pulse rate	21 C	66	50—84	112	90—124	+46	+29 — +60	+69
	23 H	59	48—75	107	90—128	+48	+30 — +66	+81
Systolic blood pressure	8 C	135	117—152	158	148—179	+23	+14 — +32	+17
	12 H	109	100—128	140	118—167	+31	+11 — +60	+28
Diastolic blood pressure	8 C	79	69—91	87	74—98	+ 8	+ 5 — + 9	+10
	12 H	67	63—71	79	61—94	+12	− 2 — +23	+18
Mean blood pressure	8 C	99	83—112	112	91—123	+14	+ 7 — +23	+14
	12 H	81	74—88	102	89—113	+21	+ 6 — +38	+26
Pulse pressure	8 C	56	37—72	71	50—81	+15	+ 5 — +23	+27
	12 H	42	36—43	61	34—90	+19	− 7 — +53	+45

Table 7 Details of changes in pulse rate and blood pressures evoked by exercise. N = number of subjects, C = control subjects, H = hypotensive subjects. Pressure values are intra-arterial and expressed in mm.Hg

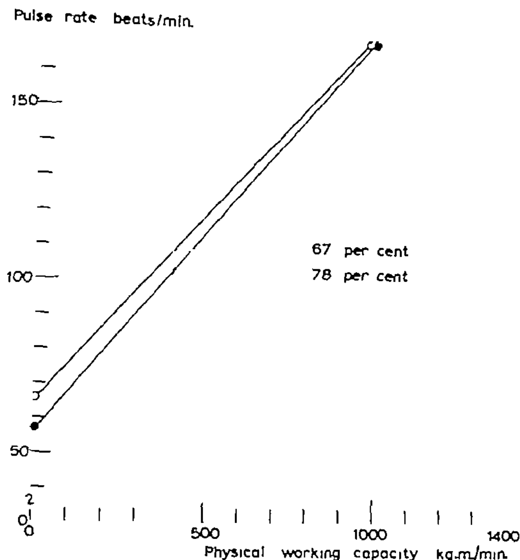


Fig 10 Graph showing mean relative increments of pulse rate at 400 kg.m./min. level in relation to mean physical working capacity of the groups. O = control group, ● = hypotensive group

indexes in the two groups, with the exception of the heart volume were grouped approximately in the same way with regard to the axes formed by the two factors. In the control group the heart volume had a tendency to increase with increasing peripheral resistance whereas in the hypotensive group the *factorial loading of the heart* volume was insignificant.

It must be realized, however that in hemodynamic studies the factor analysis is partly hampered by the fact that only a few parameters are independent, most of them being calculated from each other

III. Hemodynamic parameters during exercise

All the values were registered at a uniform load of 400 kg.m./min. between the sixth and seventh minute of exercise.

Group data are given in tables 7 & 8 and the individual values in tables 19 & 20 in the Appendix.

Pulse rate, beats/min. The mean increase was 46 in the control cases, with individual variations from 29 to 60. The mean percentile increment was 69 with a range of 39—108 per cent (table 7).

The hypotensive subjects exhibited a mean absolute increment of 48 and a percentile one of 81 per cent. The variations were from 30 to 66 and from 48 to 122 per cent, respectively. The discrepancy between the almost equal absolute increment of the pulse rate in both groups and the considerably different percentile increase is clarified in fig. 10. In this graph, lines are drawn from the pulse rates at rest to the physical working capacity values at the 170 pulse rate level. It can be seen that the percentile increments in the pulse rate at the 400 kg. m./min. level used in the exercise studies differ by 11 per cent on account of the lower starting level of the hypotensive subjects. The percentile pulse rate increments obtained from this composed graph are almost identical with the actual figures cited above obtained from the exercise hemodynamic studies. This proves the validity of the correlation between the pulse rate response to exercise and the physical working capacity.

There was no significant difference between the groups with respect to either the change or the absolute values during exercise.

Blood pressures, mm.Hg. (table 7) Distinctly undamped pressure curves were obtained in 20 cases, including 8 controls and 12 hypotensive subjects.

	N	At rest		During exercise		Change		
		Mean	Range	Mean	Range	Mean	Range	Mean %
Pulse rate	21 C	66	50—84	112	90—124	+46	+29—+60	+69
	23 H	59	48—75	107	90—139	+48	+30—+66	+81
Systolic blood pressure	8 C	135	117—152	158	148—179	+23	+14—+32	+17
	12 H	109	100—128	140	118—167	+31	+11—+60	+28
Diastolic blood pressure	8 C	79	69—91	87	74—98	+ 8	+ 5—+ 9	+10
	12 H	67	63—71	79	61—84	+12	− 2—+23	+18
Mean blood pressure	8 C	98	89—112	112	91—123	+14	+ 7—+22	+14
	12 H	81	74—86	102	89—113	+21	+ 6—+35	+26
Pulse pressure	8 C	56	37—72	71	50—91	+15	+ 5—+22	+27
	12 H	42	36—63	61	34—80	+19	− 7—+52	+45

Table 7. Details of changes in pulse rate and blood pressures evoked by exercise. N = number of subjects, C = control subjects, H = hypotensive subjects. Pressure values are intra-arterial and expressed in mm.Hg

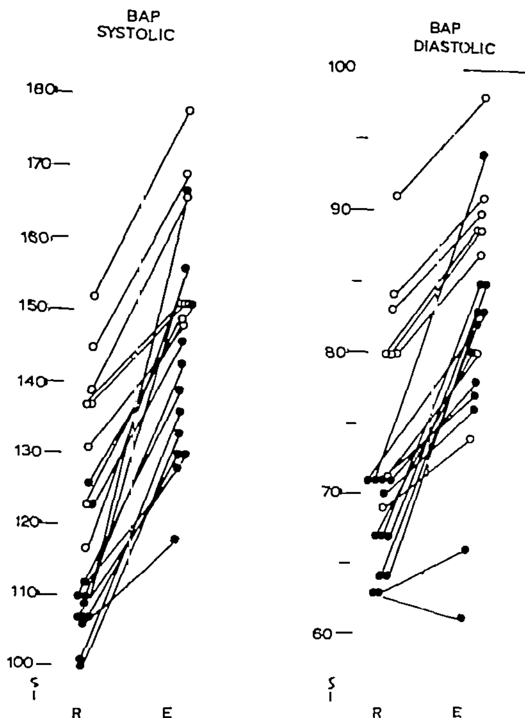


Fig. 11. Individual changes in systolic and diastolic blood pressures caused by exercise R = rest, E = exercise, O = control subjects, ● = hypotensive subjects

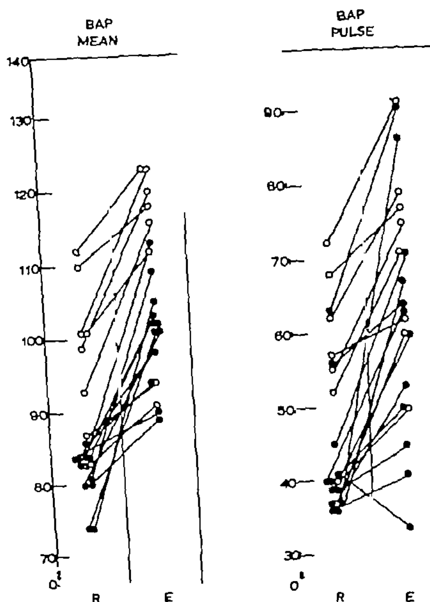


Fig. 12. Individual changes in mean and pulse pressures caused by exercise. R = rest, E = exercise, O = control subjects, ● hypotensive subjects

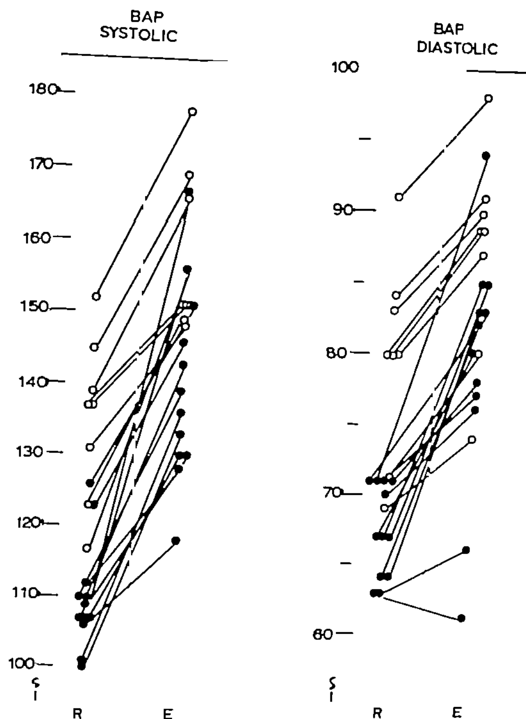


Fig. 11. Individual changes in systolic and diastolic blood pressures caused by exercise R = rest, E = exercise, O = control subjects, ● = hypotensive subjects

	N	At rest Mean	At rest Range	During exercise Mean	During exercise Range	Change Mean	Change Range	Change Mean %
Circulation time (sec.)	21 C	10.7	8.0 — 13.4	7.6	6.0 — 9.2	— 3.1	— 0.4 — — 6.8	— 29
	23 H	12.1	10.0 — 16.2	7.9	6.0 — 11.6	— 4.2	— 1.6 — — 6.0	— 35
Cardiac output (L/min.)	20 C	5.80	4.23 — 8.97	12.50	7.64 — 18.97	+ 6.61	+ 2.94 — + 11.80	+ 112
	22 H	5.15	3.00 — 6.94	11.28	8.35 — 15.43	+ 6.13	+ 2.32 — + 9.20	+ 119
Cardiac index (L/min.)	20 C	3.12	2.29 — 4.69	6.65	4.34 — 9.82	+ 3.43	+ 1.41 — + 6.09	+ 112
	22 H	2.82	2.06 — 3.81	6.21	4.21 — 8.93	+ 3.39	+ 1.82 — + 5.85	+ 119
Stroke volume (ml.)	20 C	91	57 — 130	111	64 — 148	+ 20	— 26 — + 64	+ 22
	22 H	88	64 — 110	107	80 — 140	+ 19	— 14 — + 47	+ 22
Stroke index ml.)	20 C	48.0	31.3 — 68.8	59.1	36.4 — 83.6	+ 11.1	— 13.8 — + 37.5	+ 22
	22 H	48.5	31.5 — 60.0	58.8	40.4 — 82.7	+ 10.3	— 7.3 — + 23.7	+ 22
Mean circulation time (sec.)	20 C	20.9	14.5 — 28.1	13.4	10.1 — 21.2	— 10.8	— 3.3 — — 14.6	— 36
	22 H	23.7	18.6 — 35.7	14.3	9.6 — 23.1	— 9.4	— 6.1 — — 18.8	— 39
Central blood volume (L)	20 C	2.04	1.22 — 3.17	2.78	1.35 — 3.84	+ 0.74	— 0.05 — + 1.72	+ 36
	22 H	2.11	1.14 — 2.74	2.62	1.77 — 3.86	+ 0.51	— 0.55 — + 1.51	+ 25
Central blood volume (l/m^2 BSA)	20 C	1.08	0.60 — 1.72	1.46	0.77 — 2.03	+ 0.38	— 0.03 — + 1.00	+ 36
	22 H	1.11	0.64 — 1.41	1.44	0.90 — 2.09	+ 0.33	— 0.28 — + 0.91	+ 25
Peripheral resistance (dyn.sec.cm^{-5})	7 C	1348	1063 — 1579	725	529 — 864	— 623	— 336 — — 1050	— 46
	11 H	1284	911 — 1788	702	572 — 890	— 522	— 290 — — 880	— 41
Left ventricular work index (kg.m./min./m^2 BSA)	7 C	4.1	3.4 — 5.3	10.3	6.8 — 15.1	+ 6.2	+ 3.4 — + 11.1	+ 151
	11 H	3.2	2.3 — 4.2	8.6	6.8 — 11.6	+ 5.4	+ 3.2 — + 8.0	+ 169
Left ventricular stroke work index (gm.m./m^2 BSA)	7 C	64.3	43.1 — 90.0	91.4	72.3 — 127.3	+ 27.1	+ 6.0 — + 64.0	+ 42
	11 H	52.6	35.6 — 64.1	79.7	53.7 — 111.5	+ 27.1	+ 6.4 — + 51.1	+ 54

Table 8 Details of changes in circulation times, blood flows, pressure-flow relationships, and central blood volumes evoked by exercise. N = number of subjects, C = control subjects, H = hypotensive subjects

increase of 119 per cent with the range of variation from 59 to 203 per cent. The numerical mean values of cardiac output and cardiac index in the control group during exercise were 12.50 and 6.65 respectively. The values of the hypotensive group were 11.28 and 6.21, differing insignificantly from the control figures.

Using exactly the same technique and exercise load, Frick, Konttinen & Sarajas (1961) found an average increase of 112 per cent in the cardiac index of ten young healthy recruits.

Stroke volume and stroke index, ml., (table 8) The stroke volume decreased in two control subjects, showed no change (± 5) in three cases, and increased in all the others. The average change in the whole group was + 22 per cent. A decrease occurred in one hypotensive subject, insignificant changes in four and increases in the others. The average change in the

The systolic pressure of the controls showed a mean increment of 23 and a range from 14 to 32. The percentile increase ranged between 10 and 27 with a mean of 17 per cent. In the hypotensive group the exercise evoked a mean absolute increase of 30 with a range from 11 to 60 and a mean percentile increment of 28 with a range of 10—56 per cent. The changes caused by exercise in the two groups did not differ significantly On the other hand, however the absolute values during exercise remained lower in the hypotensive group than in the controls (P < 0 01) The individual responses to exercise are illustrated by figure 11.

The diastolic pressure increased by 7—13 per cent in the control cases. The mean percentile increase was 10 per cent The absolute increase in the control group ranged between 5 and 9 and showed a mean of 8. In one hypotensive subject (case 36) the diastolic pressure decreased by 3 per cent, while all the others showed an increase of from 5 to 33 per cent mean 18 per cent. The absolute increment averaged 12, with a variation from 3 to 23 The change between the groups differed insignificantly whereas the absolute values during exercise showed a probable difference (P < 0 05) The individual responses to exercise are shown in figure 11.

The mean increase in the mean pressure was 14 in the control group and 21 in the hypotensive group The ranges were from 7 to 23 and from 6 to 38 respectively In percentages the increments in the control group varied between 7 and 25 per cent and in the hypotensive group between 11 and 51 per cent. The means were 14 and 26 per cent, respectively The increase caused by exercise was not significantly different in the groups. The same was true of the numerical values during exercise. Figure 12 illustrates the individual values.

The pulse pressure increased in all control cases by an average of 27 per cent, the individual variation being from 8 to 62 per cent. In absolute values the figures were a mean of 15 and a range from 5 to 23. One hypotensive subject (case 31) exhibited a 17 per cent decrease in the pulse pressure In the other hypotensive subjects the exercise had evoked a widening in the pulse amplitude. The mean increase in the whole group was 19 corresponding to 48 per cent. The numerical increment evoked by exercise and the absolute values during exercise did not differ in the two groups. The individual pulse pressure values are shown in figure 12.

Circulation time, sec., (table 8) The control group showed a mean value of 7 6 and the hypotensive group 7.9 thus differing insignificantly from each other In the control group the circulation time decreased by an average of 29 per cent and in the hypotensive group by 36 per cent.

Cardiac output and cardiac index, l min., (table 8) The cardiac output increased in the control subjects by 47—232 per cent, the average increment being 112 per cent. The hypotensive cases exhibited an average

	N	At rest Mean	At rest Range	During exercise Mean	During exercise Range	Change Mean	Change Range	Change Mean %
Circulation time (sec.)	21 C	10.7	8.0 — 13.4	7.6	6.0 — 9.2	−3.1	−0.4 — −6.8	−29
	23 H	12.1	10.0 — 16.2	7.9	6.0 — 11.6	−4.2	−1.0 — −6.0	−35
Cardiac output (L/min.)	20 C	5.88	4.23 — 8.97	12.50	7.64 — 18.97	+6.61	+2.94 — +11.86	+112
	22 H	5.15	3.69 — 6.84	11.28	8.35 — 15.43	+6.13	+2.32 — +9.20	+119
Cardiac index (L/min.)	20 C	3.12	2.29 — 4.69	6.65	4.34 — 9.92	+3.43	+1.41 — +6.99	+113
	22 H	2.82	2.08 — 3.61	6.21	4.21 — 8.83	+3.39	+1.82 — +5.95	+119
Stroke volume (ml.)	20 C	91	57 — 130	111	64 — 145	+20	−26 — +64	+22
	22 H	88	54 — 110	107	69 — 143	+19	−14 — +47	+22
Stroke index (ml.)	20 C	48.0	31.3 — 68.8	59.1	36.4 — 83.6	+11.1	−13.8 — +37.5	+22
	22 H	48.5	31.5 — 69.0	58.8	40.4 — 82.7	+10.3	−7.5 — +23.7	+22
Mean circulation time (sec.)	20 C	20.9	14.5 — 29.1	12.4	10.1 — 21.2	−10.8	−3.3 — −14.6	−58
	22 H	23.7	18.6 — 35.7	14.3	9.6 — 22.1	−9.4	−6.1 — −15.8	−39
Central blood volume (l.)	20 C	2.04	1.22 — 3.17	2.78	1.35 — 3.84	+0.74	−0.05 — +1.72	+38
	22 H	2.11	1.14 — 2.74	2.62	1.77 — 3.86	+0.51	−0.55 — +1.51	+25
Central blood volume (l./m. BSA)	20 C	1.08	0.69 — 1.72	1.46	0.77 — 2.03	+0.38	−0.03 — +1.00	+36
	22 H	1.11	0.64 — 1.41	1.44	0.90 — 2.09	+0.33	−0.28 — +0.91	+25
Peripheral resistance (dyn.sec.cm^{-5})	7 C	1348	1063 — 1579	725	329 — 964	−623	−338 — −1050	−46
	11 H	1284	911 — 1798	762	572 — 990	−522	−250 — −890	−41
Left ventricular work index (kg.m./min./m^2 BSA)	7 C	4.1	3.4 — 5.9	10.3	6.9 — 16.1	+6.2	+3.4 — +11.1	+151
	11 H	3.2	2.3 — 4.2	8.6	6.8 — 11.6	+5.4	+3.2 — +8.0	+169
Left ventricular stroke work index (gm.m./m^2 BSA)	7 C	64.3	49.1 — 90.0	91.4	72.3 — 127.3	+27.1	+6.0 — +64.0	+42
	11 H	52.6	35.6 — 64.1	79.7	53.7 — 111.5	+27.1	+6.4 — +51.1	+54

Table 8. Details of changes in circulation times, blood flows, pressure-flow relationships, and central blood volumes evoked by exercise. N = number of subjects, C = control subjects, H = hypotensive subjects

increase of 119 per cent with the range of variation from 59 to 203 per cent. The numerical mean values of cardiac output and cardiac index in the control group during exercise were 12.50 and 6.65, respectively. The values of the hypotensive group were 11.28 and 6.21, differing insignificantly from the control figures.

Using exactly the same technique and exercise load, Frick, Konttinen & Sarajas (1961) found an average increase of 112 per cent in the cardiac index of ten young healthy recruits.

Stroke volume and stroke index, ml., (table 8) The stroke volume decreased in two control subjects, showed no change (± 5) in three cases, and increased in all the others. The average change in the whole group was + 22 per cent. A decrease occurred in one hypotensive subject, insignificant changes in four and increases in the others. The average change in the

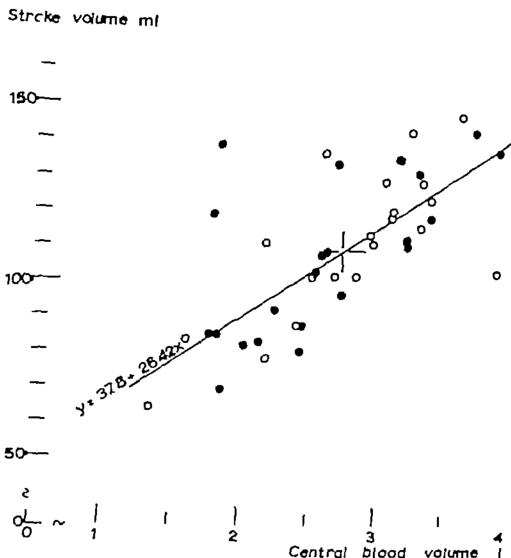


Fig. 13. Relation between stroke volume and central blood volume during exercise.
O = control subjects, ● = hypotensive subjects

hypotensive group was + 22 per cent i.e. exactly the same increment as in the control cases. The actual mean values during exercise were 111.3 for the controls and 107.0 for the hypotensive subjects, corresponding to stroke indexes of 59.1 and 58.8, respectively. Neither these values nor the change evoked by exercise differed significantly in the groups compared.

In the above cited study of Frick, Konttinen & Sarajas the stroke index increased by an average of 17 per cent.

A significant correlation with the central blood volume was found in the control group (r = 0.50) in the hypotensive group (r = 0.74) and in the total series (r = 0.74). This is illustrated by figure 13 showing the regression line for the total material.

Mean circulation time, sec., (table 8) decreased in the control cases by an average of 36 per cent, with a range

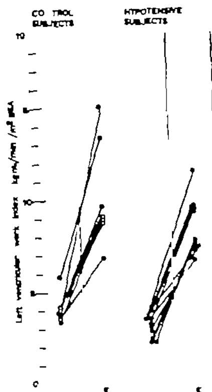


Fig. 14. Individual changes in left ventricular work index evoked by exercise. R = rest, E = exercise

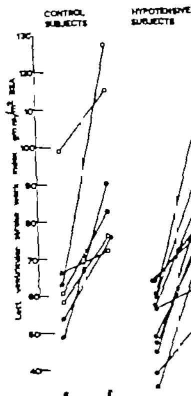


Fig. 15. Individual changes in left ventricular stroke work index evoked by exercise. R = rest, E = exercise

of variation from 15 to -54 per cent. The decrease in the hypotensive group varied between 31 and 52 per cent, with a mean of 39 per cent. The absolute decrement caused by exercise was 7.6 in the controls and 9.4 in the hypotensive subjects ($P < 0.05$). The mean figures during exercise, i.e. 13.4 for the controls and 14.3 for the hypotensive group, did not differ statistically.

Central blood volume, L or L/m² BSA, decreased by 3 per cent in one control case and increased in all the others by 11 to 119 per cent. The mean change in the whole control group was + 36 per cent. In the hypotensive group one case exhibited a 24 per cent decrease, one case unchanged values, and the others an increase of from 3 to 96 per cent. The mean increment in the hypotensive group was 25 per cent. No statistical difference was found between the groups with regard to either the change caused by exercise or the absolute values during exercise. The average for the latter was 2.78 for the controls and 2.62 for the hypotensive subjects, corresponding to 1.46 and

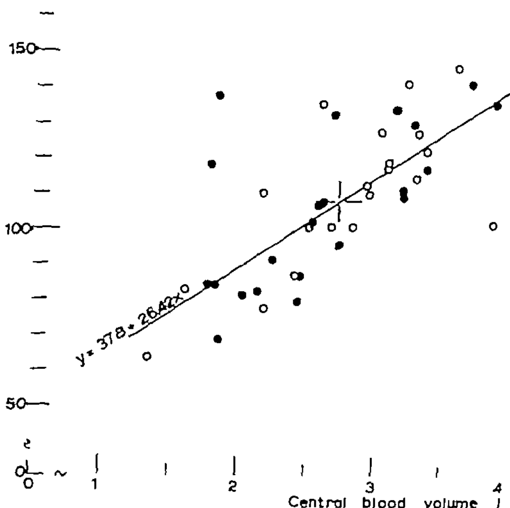


Fig 13. Relation between stroke volume and central blood volume during exercise. O = *control subjects*, ● = *hypotensive subjects*

hypotensive group was + 22 per cent, i.e., exactly the same increment as in the control cases. The actual mean values during exercise were 111.3 for the controls and 107 0 for the hypotensive subjects, corresponding to stroke indexes of 59.1 and 58 8 respectively Neither these values nor the change evoked by exercise differed significantly in the groups compared.

In the above cited study of Frick Konttinen & Sarajas the stroke index increased by an average of 17 per cent.

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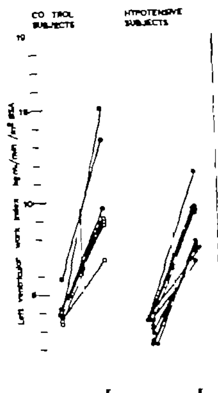


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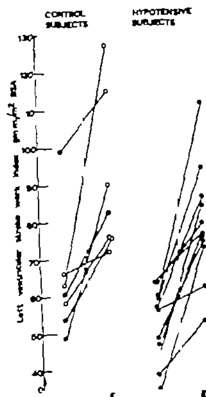


Fig. 15. Individual changes in left ventricular stroke work index evoked by exercise. R = rest, E = exercise

of variation from 15 to -34 per cent. The decrease in the hypotensive group varied between 31 and 52 per cent, with a mean of 39 per cent. The absolute decrement caused by exercise was 7.5 in the controls and 9.4 in the hypotensive subjects ($P < 0.05$) The mean figures during exercise, i.e., 13.4 for the controls and 14.3 for the hypotensive group, did not differ statistically

Central blood volume, l. or l./m² BSA, decreased by 3 per cent in one control case and increased in all the others by 11 to 119 per cent. The mean change in the whole control group was + 38 per cent. In the hypotensive group one case exhibited a 24 per cent decrease, one case unchanged values, and the others an increase of from 3 to 96 per cent. The mean increment in the hypotensive group was 25 per cent. No statistical difference was found between the groups with regard to either the change caused by exercise or the absolute values during exercise. The average for the latter was 2.78 for the controls and 2.62 for the hypotensive subjects, corresponding to 1.46 and

1.44 respectively when calculated per sq.m. of body surface area (table 8)

With 6 exceptions in the total series, the change in the central blood volume was closely paralleled by a similar trend in the stroke volume.

Peripheral resistance, dynes sec. cm $^{-5}$., decreased in all control subjects with available measurements by 28—66 per cent, mean 46 per cent, and in all hypotensive subjects with pressure-flow data by 27—53 per cent, mean 41 per cent. The mean figures during exercise were 725 for the controls and 762 for the hypotensive subjects. The difference was insignificant. The same was true of the change caused by exercise (table 8)

Left ventricular work index, kg.m./min./m ^{2} BSA, increased in control cases by 97—278 per cent, mean 151 per cent. The corresponding increment in the hypotensive group was 169 per cent, with variations ranging from 8 to 243 per cent (table 8) The mean value of the hypotensive subjects during exercise 8.62, did not differ statistically from the control mean, 10.29 The same holds as well for the change caused by exercise in the compared groups. The individual changes are illustrated by fig. 14.

Left ventricular stroke work index, gm.m./m ^{2} BSA, (table 8) The mean values of the controls and the hypotensive subjects during exercise i.e., 91.4 and 79 7 respectively did not differ statistically The mean change was + 42 per cent in the controls and + 54 per cent in the hypotensive cases. The change in the two groups differed insignificantly The individual responses are shown in fig. 15

DISCUSSION

General findings

In the anthropometric parameters the only real difference was found in body weight. The lower mean body weight of the hypotensive group does not differ however more than by 0.1 kg. from the mean of 275 Finnish recruits studied by Frick & Halonen (1961) and by 0.9 kg. from the mean of 204 Swedish conscripts studied by Hallström (1961) The fact that the mean age of the soldiers in the latter two series was some 3—4 years lower can hardly be of significance in smoothing out the difference towards the hypotensive group. It is therefore evident that the hypotensive group in the present series does not differ from a larger population series with regard to body weight. Nevertheless, this does not exclude the fact that the hypotensive series included several subjects of a rather lean build. Case 22, a man with a stature of 175 cm. weighing 53 kg., may serve as an example.

It is generally considered that hypertension is more frequent among obese persons (e.g., Scirk 1957) and that hypotension is often combined with asthenic habitus (e.g., Pierach & Heynemann 1959) The occurrence of the latter combination is not directly confirmed by the present observations. The series, nevertheless, is too small for any frequency studies. It is also likely that the distribution of the body build in a series consisting of clinical cases is different from that in the present series.

It has been stated that there are no characteristic findings in the electrocardiograms of hypotensive subjects (Pierach & Heynemann 1959) This statement is fully corroborated by the present findings. Apart from a slow pulse rate in several cases, nothing definitely abnormal could be found. The frequency of partial right bundle branch block was high, but this pattern occurs frequently especially in young adults who are otherwise normal (Sodi-Pallares & Calder 1956) Neither can any special significance be attached to the frequent occurrence of precordial S—T elevations, since absence of this elevation was observed in only 8.8 per cent of a large population series (Hiss, Lamb & Allen 1960) In addition to the study just cited, in which the greatest S—T elevation was 0.5 mv elevations up to 3 mv were rather often found by Sokolow & Friedlander (1949)

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vations of Pellegrini's group (1952, 1955). The in general, 1—3 cm. shorter transversal diameters of hypotensive hearts as compared with control cases observed by Martini & Pierach (1926) and Barsieck (1943) are merely to be interpreted as a criterion of the heart shape and not directly of the volume. Fully realizing the inconsistency between the electrocardiographic and the anatomic heart positions it may be concluded that in the present series the hypotensive group included several hearts in a vertical position. As a corollary to this line of reasoning is the more frequent occurrence of the so-called "drop" hearts in the hypotensive group than among the control subjects. The "drop" heart is one of the characteristic findings in hyposphygmia (Luisada 1948). Other criteria of this subgroup of hypotension i.e. extreme reduction of the pulse pressure and orthostatic tachycardia and hypotension, were not found in the present study.

With a view to the task of the blood to transport oxygen, the insignificantly lower total blood volume of the hypotensive group contained a relatively higher red cell volume and hemoglobin concentration than that of the control group. A reduced total volume of blood as reason for the low blood pressure can be excluded in the present series. A normal blood volume in hypotensive subjects was also found by Pellegrini's group (1952, 1955).

In view of the descriptions in the literature which usually portray the hypotensive subject as an individual with a lack of physical stamina and endurance (e.g., Pennock 1957) the normal mean physical working capacity of the hypotensive group in the present series is worth noting. No actual reference to the physical fitness of hypotensive subjects is to be found in the literature. Judging from the recovery rate of the pulse frequency after exercise it may be assumed that the subjects studied by Reindell (1949) and in part those examined by Siedek, Wenger & Hörtnagel (1950) were characterized by poor physical fitness, whereas the hypotensive students studied by Thacker (1940 b) were in the same physical condition as the normotensive ones.

The question now arises whether a poor physical fitness can be generalized as being a prominent feature of the hypotensive population. No trend is evident when the present series is analyzed case by case with regard to the correlation between the degree of hypotension and the physical working capacity. From the information derived from the present data it may be sufficiently reliable to assume that poor physical fitness is not a reflection of essential hypotension but may be correlative to the rather lean body build of some hypotensive subjects. Further it is evident that just this type of hypotensive individuals are in majority in series selected on clinical grounds.

Hemodynamics at rest

One of the most salient findings was the slow pulse rate in several hypo-

tensive subjects. If 60 beats/min. is regarded as the limit for bradycardia (Katz & Pick 1956, Schütz 1958) 52 per cent of the hypotensive subjects were bradycardiacs and even the mean pulse frequency of the whole hypotensive group passed just below this limit. This is in conformity with the findings of most workers in the field but in contrast to the observations of Pellegrini's group (1952, 1955). This difference may arise from apprehension due to cardiac catheterization used by the Italian authors. Besides the role of this bradycardic pulse in haemodynamics, certain practical aspects of blood pressure measurements by the cuff method become evident. Comparisons of cuff and intra-arterial pressures in the present series showed that the systolic cuff pressure agrees fairly well and the diastolic pressure with reasonable accuracy with the intra-arterial pressures. However calculation of the mean pressure by halving the sum of the systolic and diastolic pressures or by adding a certain fraction of the pulse pressure to the diastolic pressure may lead to an erroneus result when bradycardic individuals are studied. The relatively long duration of the diastole lowers the mean pressure, often to a level quite near the diastolic pressure. In consequence in the earlier studies, in which the cuff method has been used, the hemodynamic parameters referred to the mean pressure may be erroneous.

Normal stroke volumes were found in nearly all hypotensive subjects. In only two cases was the stroke volume smaller than the lowest figure in the control group (cases 31 and 36). These two cases exhibited also relatively rapid heart rates as compared to the rest of the group. Obviously therefore the smaller cardiac output characterizing the hypotensive group was a result of the slow pulse rate. The significance of the bradycardic pulse is illustrated by figure 16, which shows the relation between the pulse rate and the cardiac index (controls $r = 0.23$, hypotensives $r = 0.40$ total series $r = 0.39$ $P < 0.01$).

The question of the physiological and/or pathological significance of the low cardiac output is an intricate one. The voluminous literature covering the topic of hemodynamics in a trained person contains diverging views concerning the total blood flow. Berger & Ollos (1933) found a small mean cardiac output in trained persons, mediated by both a bradycardic pulse and a small stroke volume. The existence of this combination was confirmed by Mellerowicz (1956) in an extensive study on top-class endurance athletes. Muschhoff, Reindell & Klepzig (1959) also, found a smaller cardiac output at rest in athletes than in non-athletes. The stroke volumes, however were equal, but the pulse rates were lower in athletes. Contrary to the above Henderson, Haggard & Dolley (1927) found a larger mean cardiac output at rest in athletes than in untrained subjects. Concerning the stroke volume, however several authors have shown that an athlete has a larger stroke volume at rest than an untrained person (e.g., Bock et al. 1928

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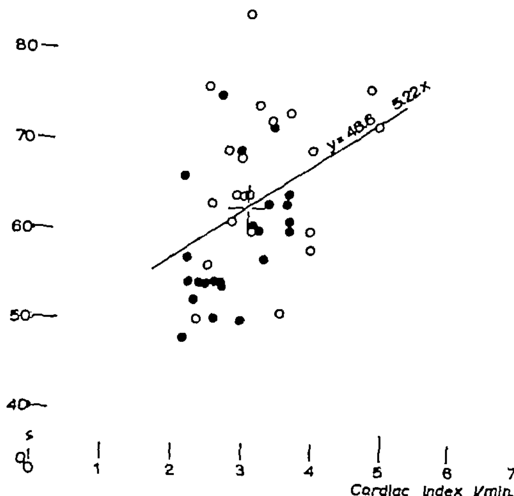


Fig. 16. Relation between pulse rate and cardiac index, O = control subjects, ● = hypotensive subjects

Christensen 1931 Muschoff *et al.* 1957) An increase in stroke volume can also be observed in sedentary workers during a training program (Frick, *Konttinen & Sarajas* 1961)

It is therefore evident that, inspite of the bradycardic pulse the low cardiac output of the hypotensive group in the present investigation cannot be traced to a trained physique. This is already obvious from the data on heart volume and physical working capacity It is likewise evident that the small total blood flow of the hypotensive subjects can hardly be inter preted as *pathological, at least not* to such degree as to be capable of *lowering* the physical fitness of these subjects. In the absence of all pathological evidence it would neither be logical to interpret the low cardiac output as being on the descending

limb of a Starling curve The present data rather warrant the deduction that the low cardiac output of the hypotensive subjects is in the majority of cases due to an increased vagal tone, leading to infrequent discharge of the sinus node.

All the values of calculated peripheral resistance in the hypotensive group were within the limits observed in the control subjects. Thus it may be concluded that the significantly lower mean pressure of the hypotensive group was due to the low output. While this holds for the entire group, each individual case may show various output resistance interrelations in the combinations offered by two parameters with an about 100 per cent normal range. The dependence of the low pressure from a low cardiac output is the counterpart of the cardiogenic hypertension (Freis 1960) in which the elevated blood pressure is due to increased total blood flow against normal peripheral resistance (Werkö & Lagerlöf 1949· Varnauskas 1955 Widimský Fejfarová & Fejfar 1957)

In addition to the small cardiac output, the slightly lower central elasticity in the hypotensive group may be of significance in influencing the pressure oscillations. This is illustrated by correcting the pulse pressure according to central elasticity as a result of which the difference between the pulse pressures in the groups was reduced. The method used in the present investigation to obtain estimates of the central elasticity may be *criticized*, but neither is the application of the pulse wave velocity to determination of the elasticity free from inaccuracy arising from correction factors due to the nonlinearity of the pressure-volume curves of the human aorta (Hamilton 1953 Shock 1961) The present findings also bring out the lack of correlation between stroke volume and pulse pressure in some instances. The same deviation was emphasized by Wiggers (1938) in an analytical survey on hypertension.

Both the total and the stroke work of the left ventricle were low in the hypotensive group. Considering the work per minute of the left heart, both determinants, i.e., volume and pressure, were low whereas the low stroke work was due only to the low mean systemic pressure. The reducing effect of the low pressure-volume combination on the left heart pressure work is illustrated in figure 17 from which it can be clearly seen that the hypotensive subjects exhibited a lower left ventricular work index than the control subjects against the same peripheral resistance. Referring to the evidence accumulated from experiments with heart-lung preparations, an increase of arterial pressure leads to a greater increase in myocardial oxygen consumption than an increase in cardiac output (*Katz 1954, Katz, Katz & Williams 1955*· Sarnoff *et al.* 1958) Further when the output and the aortic pressure are kept constant, the heart rate has been found to be an independent variable in increasing the myocardial oxygen consumption (Laurent *et al.* 1956· Braunwald *et al.* 1958) The best correlation has been

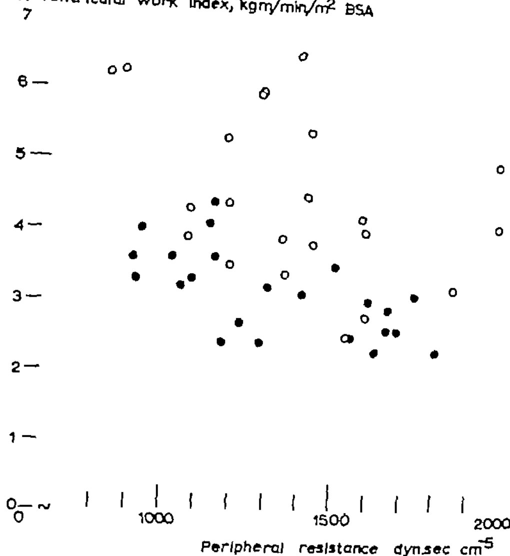


Fig 17 Relation between left ventricular work index and peripheral resistance. O — control subjects, ● = hypotensive subjects

found to exist between the myocardial oxygen consumption and tension time index (Sarnoff *et al.* 1958) or the latter s analogue ejection pressure-time (Katz 1958) both indexes consisting of pressure and rate When these data are employed to clarify the significance of the low pressure work indexes of the hypotensive subjects, it is obvious that these low output hearts utilize a smaller fraction of the total oxygen for the whole organism than the hearts of the reference group. This is not only because of the smaller volume work, which probably is of minor significance as a metabolic cost but preponderantly on account of the low mean systemic pressure and low pulse rate. In this respect the hypontensive heart resembles the athlete s heart,

but has in addition the advantage of a normal stroke volume and evidently also a lower mean pressure than the latter as can be judged from the study of Mellerowicz (1956)

Thus the same finding of a low left heart work in hypotensive subjects has been made in the present study as earlier by Martini & Pierach (1926) However the interpretation of this event is quite the opposite.

Hemodynamics during exercise

The marked difference in the pulse rates at rest was completely abolished during exercise. This change is attributable to sympathetic stimulation known to occur during exercise and is concordant with the vagus-sympathicus interrelations in cardiac control experimentally shown by Rushmer (1958) While the mean absolute increment of the pulse rate evoked by exercise was about the same in both of the groups, the percentile increments were quite different, due to the lower starting level in the hypotensive group. Because the same load was used in all the exercise experiments, the absolute change produced by this load was considered more informative than the relative change (Grosse—Brockhoff, personal communication) The same rule applies to all the parameters examined during exercise.

The behavior of the pressure parameters during exercise in both of the groups is similar to that found by Eskildsen, Götsche & Hansen (1950) Donald et al. (1955) and Holmgren (1956) in normal subjects. The blood pressure response to a work of 600—700 kg.m./min. on an ergometer or to 40 kneelings was the same in the hypotensive subjects as in the controls studied by Pellegrini (1952)

The calculated peripheral resistance decreased in all hypotensive and control subjects. This is in conformity with the findings in normal (Donald et al. 1955) and hypertensive subjects (Varnauskas 1955 Taylor Donald & Bishop 1957 Varnauskas et al. 1961) Thus the increase of the cardiac output in relation to the blood pressure response was substantially the same in both of the groups. A normal response of the cardiac output to exercise in hypotensive subjects was also observed by Pellegrini (1952) whereas Raindell (1949) found in general a smaller increment of the cardiac output during exercise in hypotensive subjects than in his control cases. The circulatory response to exercise at a given load is certainly correlative to the physical fitness. This correlation fits in most clearly with the pulse rate response to exercise, which in the present study was illustrated by figure 10 Varying results have been obtained for the behavior of the cardiac output during exercise. The total blood flow of athletes has been reported to be either lower (Krogh & Lindhard 1912, Christensen 1931) or higher (Henderson, Haggard & Dolley 1977 Bock et al. 1928, Peterson et al. 1948 Musschoff, Reindell & Kleptig 1959) than that of non-athletes at the same given level of exercise. This difference is to be traced back, at least in part, to the differing behavior of the stroke volume

during exercise. In the present study in which the physical working capacity was almost the same in both groups, almost identical increments of the cardiac output were found in the groups during exercise However this increment could not be predicted in each individual case on basis of the value of physical working capacity because of various rate-stroke volume interrelations during exercise

While the total blood flow was mainly augmented by an increased pulse rate most of the subjects in both of the groups exhibited also an increased stroke volume. The response to exercise was in this respect similar in both of the groups. The significant correlation between the central blood volume and the stroke volume, observed also at rest, confirms the earlier observations of Nowy & Frings (1958) and demonstrates the role of the central blood volume as a determinant of the stroke output. With few exceptions the central blood volume increased during exercise in both of the groups. These values for the different groups are comparable, but it cannot be excluded that raising of the feet to the ergometer pedals about 20° above the horizontal level before starting the exercise experiments may have some significance in enhancing the venous return and altering the blood distribution, with a possible increase in the central blood volume. The same reservation was made by Eliasch (1952) in studies of mitral stenosis. Consequently the data on stroke volume during exercise are not strictly valid to warrant a discussion of the effect of exercise on stroke output in general.

Left ventricular pressure work increased in a similar manner and amount in control and hypotensive subjects. The metabolic benefit of a slow pulse and a low pressure at rest in hypotensive subjects was no longer demonstrable This is confirmatory evidence for a non-athletic circulatory adjustment in hypotensive subjects, already obvious from the hemodynamic data at rest.

Clinical correlations

Orthostatic and postural hypotension are usually easily distinguishable from essential hypotension by the pathological response of the blood pressure on tilting the subject. The supine resting blood pressure is of no significance in orthostatic or postural hypotension, although low values have occasionally been reported (Bickelman, Lippschutz & Brunjes 1961) While the reduced cardiac output during tilting is responsible for the cardiovascular manifestations in orthostatic and postural hypotension, it has been demonstrated that the principal fault lies in failure of the peripheral vasoconstrictive mechanisms (Hickam & Pryor 1951 Bickelman Lippschutz & Brunjes 1961) due to weakness of the reflectory mechanisms in the former and to a greater loss of autonomic nervous function in the latter (Judson 1953) A low 24-hour urinary excretion of norepinephrine has been observed in postural hypotension by Luft & v Euler (1953) and

Benestad & Bøe (1955) Further the normal increase in the plasma norepinephrine level during tilting fails to occur in subjects with postural hypotension (Hickler *et al.* 1959) but does occur in cases of orthostatic hypotension (Sundin 1956) In essential hypotension the plasma epinephrine levels have been found to be normal (Pellegrini 1952)

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Holmgren *et al.* (1957 a, b) described a state characterized by high orthostatic pulse rates, low physical working capacity and an elevated cardiac output at rest, defined as vasoregulatory asthenia. Rapid pulse rates at rest were also observed in these subjects. One of the 12 cases presented had a low blood pressure

It appears that essential hypotension represents the first part of the blood pressure distribution curve in a population. It may coincide with several clinical states in relation to its frequency In this respect it is the counterpart of the Pickering concept of high blood pressure (1955) but when the latter is pathogenetically due to a decreased cross-sectional area of the peripheral vascular bed, essential hypotension seems in the majority of cases to be due to a low output of the heart.

during exercise In the *present study* in which the physical working capacity was almost the same in both groups, almost identical increments of the cardiac output were found in the groups during exercise However this increment could not be predicted in each individual case on basis of the value of physical working capacity because of *various rate-stroke volume* interrelations during exercise.

While the total blood flow was mainly augmented by an increased pulse rate most of the subjects in both of the groups exhibited also an increased stroke volume. The re*sponse to exercise was in this respect* similar in both of the groups. The significant correlation between the central blood volume and the stroke volume observed also at rest, confirms the earlier observations of Nowy & Frings (1958) and demonstrates the role of the central blood volume as a determinant of the stroke output. With few exceptions the central blood volume increased during exercise in both of the groups. These values for the different groups are comparable but it cannot be excluded that raising of the feet to the ergometer pedals about 20° above the horizontal level before starting the exercise experiments may have some significance in enhancing the venous return and altering the blood distribution, with a *possible increase in the* central blood volume. The same reservation was made by Eliasch (1952) in studies of mitral stenosis. Consequently the data on stroke volume during exercise are not strictly valid to warrant a discussion of the effect of exercise on stroke output in general.

Left ventricular pressure work increased in a similar manner and amount in control and hypotensive subjects. The metabolic benefit of a slow pulse and a low pressure at rest in *hypotensive subjects was no longer* demonstrable This is confirmatory evidence for a non-athletic circulatory adjustment in hypotensive subjects, already obvious from the hemodynamic data at rest.

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hypotensive subjects ranged between 911 and 1798 dynes sec. cm^{-5} and was within the values observed in the controls. The hemodynamic significance of the low cardiac output is discussed.

A significant correlation between the stroke volume and the central blood volume was found in both groups. In spite of similar stroke volumes the hypotensive subjects had a smaller pulse pressure than the controls. The central elasticity was slightly lower in the hypotensive group.

The left ventricular stroke work was clearly lower in the hypotensive group than in the controls. The mean left stroke work index was 53.3 gm.m./m^2 BSA for the hypotensives and 67.0 gm.m./m^2 BSA for the controls. The total left ventricular pressure work of the hypotensives was distinctly low the mean being 3.1 kg.m./min./m^2 BSA, against 4.4 kg.m. min./m^2 BSA in the control group.

During the exercise test, consisting of 6 to 7 minutes ergometer pedalling in the supine position at a load of 400 kg.m. min., the systolic and diastolic pressures of the hypotensive subjects were still lower than the pressures in the control group. The values for the total blood flow stroke output, central blood volume, peripheral resistance and left heart pressure work did not differ. The change caused by the exercise was also similar in both groups. It consisted of an increase in both the pulse rate and the stroke volume, the former however being the main determinant increasing the total blood flow. The approximately 20 per cent increase in the stroke volumes was closely paralleled by a similar increase in the central blood volume resulting in a significant correlation between the stroke volume and the central blood volume also during exercise. The exercise evoked an average increase of 112 per cent in the output of the controls and one of 119 per cent in the hypotensives. The peripheral resistance fell in both groups by more than 40 per cent.

The findings are discussed in the light of earlier observations on essential hypotension and of the concepts of cardiogenic hypertension and essential hypertension.

SUMMARY

Twenty three men with essential hypotension, i.e. systolic blood pressure of 110 mm. Hg or less, who were volunteers from large industrial plants, were studied to obtain information on the hemodynamics of persistently low blood pressure. In addition to the hemodynamical studies proper the body build, electrocardiographic findings, blood volume heart volume from routine chest x rays, and the physical working capacity were recorded to obtain an insight into the hypotensive population. Twenty-one healthy normotensive men served as controls.

The brachial arterial pressure was recorded *directly via an indwelling* needle in the artery The cardiac output and central blood volume were obtained with the dye dilution and ear oximeter technique utilizing Evans blue as indicator The total peripheral resistance, central elasticity and left heart pressure work were calculated according to current formulas using the pressure and flow data. The validity of the ear oximeter technique was tested against the multiple sampling method and a good agreement was found. The circulatory parameters were also registered during supine ergometer work.

The hypotensive subjects were found to be of *slightly lighter body weight* but otherwise to possess a similar body build as the control subjects. The mean *total* blood *volume of the* control group was 2921 ml. per square meter of body surface The value for the hypotensive group was 2859 ml. The heart volume of the hypotensive group was comparable with that of the controls. The mean physical working capacity was normal.

Comparisons between intra-arterial and cuff pressure values showed that the low blood pressure was not cuff hypotension in spite of a smaller arm circumference in the hypotensive subjects.

The hemodynamics at rest was characterized in several hypotensive subjects by a slow pulse rate The mean pulse rate of the hypotensive group was below 60 beats per minute. The mean cardiac output of 5.11 l. min. and the cardiac index of 2.81 l min. in the hypotensive group were clearly lower than the reference points, i.e., 6.04 and 3.21 l./min The stroke volumes and the stroke volumes per square meter of body surface of the hypotensive individuals were in general in the normal range The calculated peripheral resistance of the

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APPENDIX

Tables of Individual Values

Table 9. Anthropometric data of control subjects. Weight in kg., height, biacromial diameter, bicristal diameter, and arm circumference in cm. BSA = body surface area in sq.m.

Case number	Age	Weight	Height	BSA	Biacr. diam.	Bicrist. diam.	Arm circumf.
1	30	84.0	184	2.06	41.5	25.0	32.0
2	25	83.0	186	2.06	38.0	30.5	32.0
3	26	65.0	168	1.71	38.0	28.5	33.5
4	20	68.5	172	1.80	39.5	28.5	29.0
5	19	73.0	187	1.89	41.0	28.5	28.5
6	23	67.0	171	1.78	39.0	29.0	28.5
7	20	91.0	181	2.08	43.0	29.0	32.0
8	19	68.0	178	1.82	38.5	28.0	28.5
9	26	67.0	170	1.75	37.0	28.5	28.5
10	20	72.0	175	1.84	40.0	28.5	29.0
11	19	74.0	181	1.91	42.0	28.5	27.0
12	20	77.0	183	1.95	40.0	27.5	31.5
13	22	81.0	181	1.99	40.0	30.0	32.0
14	25	79.0	184	1.98	39.5	31.0	29.0
15	24	65.5	178	1.80	39.5	25.0	26.5
16	23	83.0	186	2.04	41.0	29.0	30.5
17	20	72.0	178	1.85	38.0	28.0	29.0
18	23	67.0	172	1.76	39.0	29.0	30.0
19	20	72.0	181	1.89	42.0	28.5	27.5
20	24	64.5	172	1.75	39.0	25.0	26.5
21	20	80.0	180	1.97	41.5	27.5	31.0

Table 10. Anthropometric data of hypotensive subjects. Weight in kg., height, biacromial diameter, bicristal diameter and arm circumference in cm. BSA = body surface area in sq.m.

Case number	Age	Weight	Height	BSA	Biacr. diam.	Bicrist. diam.	Arm circumf.
22	24	58.0	173	1.69	37.0	28.0	25.5
23	28	60.5	166	1.66	35.0	27.5	27.0
24	20	65.0	175	1.77	40.0	27.0	28.0
25	25	71.5	181	1.88	39.5	28.0	30.5
26	20	61.0	166	1.68	36.0	25.0	27.5
27	26	61.0	170	1.69	34.5	32.5	25.0
28	23	62.0	173	1.73	39.5	26.5	25.5
29	20	70.0	179	1.85	40.0	30.5	28.5
30	24	80.0	177	1.96	38.0	30.0	32.0
31	35	70.0	169	1.79	35.0	27.5	30.0
32	28	84.5	185	2.08	40.5	32.0	30.5
33	26	71.0	171	1.80	38.0	28.5	28.0
34	24	60.5	177	1.75	39.5	28.5	25.0
35	20	80.0	181	1.98	37.5	28.0	31.0
36	37	52.0	168	1.57	36.0	28.0	23.5
37	19	76.0	177	1.90	38.0	29.0	30.5
38	25	78.0	180	1.95	39.0	29.0	29.5
39	29	72.0	181	1.88	38.0	30.5	27.5
40	22	70.0	183	1.87	38.0	30.0	28.5
41	23	67.0	181	1.84	39.0	30.5	28.5
42	21	63.5	173	1.74	41.0	28.0	27.0
43	22	76.0	190	1.99	38.5	31.0	29.5
44	25	78.0	180	1.97	38.0	28.0	30.0

Table 13. Hematological data of control subjects. Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume, BSA = body surface area

Case number	Hb (g./100 ml.)	Hct (vol. %)	PV (ml.)	RCV (ml.)	TBV (ml.)	TBV/m².BSA (ml.)
1	13.3	41.9	3457	2320	6002	2913
2	14.8	44.6	3069	2561	5630	2733
3	14.0	42.1	2464	1782	4256	2489
4	13.7	44.3	2389	1900	4289	2353
5	12.0	36.5	3341	1945	5286	2799
6	15.2	47.2	2014	1803	3817	2169
7	12.8	39.5	3418	2231	5649	2716
8	12.5	38.2	3909	2416	6325	3475
9	12.4	43.3	3106	2373	5479	3131
10	12.5	41.3	3021	2126	5147	2797
11	12.0	39.0	4393	2809	7202	3771
12	13.5	42.3	3500	2566	6066	3111
13	13.7	44.3	3476	2764	6240	3136
14	11.0	38.8	3472	2362	5834	2946
15	14.1	40.6	3244	2163	5407	3004
16	15.5	44.4	4470	3368	8038	3940
17	12.4	36.0	3322	1875	5197	2809
18	14.8	42.0	2809	2034	4843	2732
19	13.3	43.9	3174	2484	5658	3009
20	12.8	41.1	2808	1959	4767	2724
21	13.6	44.9	2755	2245	5000	2538

Table 14. Hematological data of hypotensive subjects. Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume, BSA = body surface area

Case number	Hb (g./100 ml.)	Hct (vol. %)	PV (ml.)	RCV (ml.)	TBV (ml.)	TBV/m².BSA (ml.)
22	14.0	45.6	2844	2384	5228	3023
23	11.1	43.5	2760	2125	4885	2943
24	12.8	42.3	2880	2111	4991	2819
25	13.1	42.5	2960	2188	5148	2738
26	14.0	47.4	2692	2426	5118	3063
27	12.9	43.5	2556	1967	4522	2076
28	15.2	48.9	3253	2873	6126	3541
29	12.5	40.7	3447	2366	5813	3142
30	13.2	41.8	3052	2192	5244	2876
31	13.7	46.3	2159	1861	4020	2259
32	13.8	43.8	2683	2093	4776	3019
33	16.4	44.9	2867	2412	5279	2933
34	14.5	44.9	3036	2473	5509	3341
35	11.8	41.3	3894	2728	6622	3344
36	12.1	34.8	2372	1222	3594	2283
37	12.4	40.5	4143	2830	6983	3065
38	12.9	43.0	3537	2068	6205	3182
39	14.7	45.6	2576	2159	4735	2159
40	15.3	48.4	2804	2442	5046	2898
41	12.9	40.4	3420	2334	5753	3127
42	13.3	44.4	2589	2063	4658	2878
43	12.8	45.3	2179	1804	3983	2003
44	12.9	42.9	3476	2615	6081	3172

Table 11. Electrocardiographic findings of control subjects. $P-Q_c$ = heart rate corrected $P-Q$ time, $Q-T_c$ = heart rate corrected $Q-T$ interval. P.R.BBB = Partial right bundle branch block

Case number	$P-Q$	$P-Q_c$	$Q-T$	$Q-T_c$	Heart position	Precordial $S-T$ elevation	Remarks
1	0.16	0.16	0.34	0.35	semivert.	—	
2	0.15	0.15	0.35	0.39	intermed.	—	
3	0.14	0.17	0.35	0.35	intermed.	—	
4	0.16	0.16	0.35	0.37	vertical	+	
5	0.20	0.21	0.41	0.35	vertical	—	
6	0.14	0.15	0.32	0.36	intermed.	—	P.R.BBB
7	0.14	0.14	0.32	0.38	semivert.	—	
8	0.16	0.16	0.36	0.38	semivert.	—	P.R.BBB
9	0.16	0.16	0.34	0.37	vertical	—	P.R.BBB
10	0.12	0.17	0.38	0.37	semivert.	—	
11	0.18	0.14	0.30	0.37	semivert.	—	
12	0.13	0.14	0.32	0.38	semivert.	—	
13	0.16	0.16	0.34	0.36	semihoriz.	—	P.R.BBB
14	0.30	0.16	0.36	0.38	intermed.	+	
15	0.15	0.19	0.40	0.37	semivert.	—	
16	0.19	0.20	0.40	0.35	semivert.	—	
17	0.17	0.16	0.37	0.40	semivert.	—	
18	0.14	0.14	0.35	0.42	semivert.	—	
19	0.16	0.15	0.36	0.40	semivert.	—	
20	0.14	0.17	0.35	0.35	intermed.	+	
21	0.18	0.16	0.36	0.37	semivert.	+	

Table 12. Electrocardiographic findings of hypotensive subjects. $P-Q_c$ = heart rate corrected $P-Q$ time, $Q-T_c$ = heart rate corrected $Q-T$ interval. P.R.BBB = Partial right bundle branch block

Case number	$P-Q$	$P-Q_c$	$Q-T$	$Q-T_c$	Heart position	Precordial $S-T$ elevation	Remarks
22	0.18	0.16	0.35	0.38	vertical	+	P.R.BBB
23	0.22	0.19	0.42	0.38	vertical	+	
24	0.18	0.17	0.40	0.40	semivert.	+	
25	0.20	0.17	0.36	0.36	semivert.	—	
26	0.18	0.16	0.38	0.38	vertical	+	
27	0.15	0.16	0.37	0.39	semivert.	—	
28	0.16	0.16	0.36	0.38	intermed.	+	P.R.BBB
29	0.11	0.19	0.42	0.37	semivert.	+	
30	0.17	0.16	0.38	0.38	vertical	+	
31	0.15	0.15	0.38	0.41	intermed.	+	
32	0.16	0.16	0.38	0.40	intermed.	—	P.R.BBB
33	0.18	0.16	0.36	0.38	vertical	—	
34	0.14	0.18	0.35	0.33	semivert.	—	
35	0.15	0.17	0.34	0.34	intermed.	—	
36	0.17	0.15	0.32	0.37	semivert.	—	P.R.BBB
37	0.14	0.18	0.37	0.35	semivert.	+	
38	0.14	0.19	0.39	0.36	semivert.	+	P.R.BBB
39	0.20	0.18	0.38	0.36	semivert.	+	P.R.BBB
40	0.21	0.16	0.34	0.36	intermed.	—	
41	0.20	0.16	0.32	0.35	semihoriz.	—	
42	0.13	0.16	0.26	0.37	semivert.	—	
43	0.13	0.16	0.37	0.38	vertical	—	
44	0.14	0.18	0.39	0.37	semivert.	+	P.R.BBB

Table 13. Hematological data of control subjects. Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume, BSA = body surface area

Case number	Hb (g./100 mL.)	Hct (vol. %)	PV (mL.)	RCV (mL.)	TBV (mL.)	TBV/m²BSA (mL.)
1	13.3	41.9	3487	2520	6002	2913
2	14.8	44.6	3069	2561	5630	2733
3	14.0	42.1	2464	1792	4256	2439
4	13.7	44.3	2389	1900	4289	2383
5	12.0	34.8	3341	1945	5286	2709
6	15.2	47.2	2914	1903	4817	2189
7	12.8	39.5	3418	2231	5649	2716
8	12.5	38.2	3909	2416	6325	3475
9	12.4	43.3	3106	2373	5479	3131
10	12.5	41.3	3021	2126	5147	2797
11	12.0	39.0	4393	2809	7202	3771
12	13.5	42.3	3500	2568	6068	3111
13	13.7	44.3	3476	2764	6240	3136
14	11.9	36.8	3472	2362	5834	2946
15	14.1	40.9	3244	2163	5407	3004
16	15.5	44.4	4470	3568	8038	3940
17	12.4	38.0	3322	1875	5197	2809
18	14.8	42.0	2809	2034	4843	2732
19	13.3	43.9	3174	2484	5658	3009
20	12.8	41.1	2808	1959	4767	2724
21	13.6	44.9	2755	2245	5000	2538

Table 14. Hematological data of hypotensive subjects. Hb = hemoglobin, Hct = hematocrit, PV = plasma volume, RCV = red cell volume, TBV = total blood volume, BSA = body surface area

Case number	Hb (g./100 mL.)	Hct (vol. %)	PV (mL.)	RCV (mL.)	TBV (mL.)	TBV/m²BSA (mL.)
22	14.0	45.6	2844	2384	5229	3063
23	11.1	43.5	2760	2125	4885	2943
24	12.8	42.3	2850	2111	4901	2819
25	13.1	42.5	2960	2188	5148	2738
26	14.0	47.4	2692	2426	5118	3083
27	12.9	43.5	2555	1967	4522	2678
28	15.2	46.9	3253	2873	6188	2541
29	12.5	40.7	3447	2366	5813	3142
30	13.2	41.8	3032	2182	5244	2676
31	13.7	46.3	2159	1861	4020	2259
32	13.8	43.8	2695	2033	4778	2319
33	16.4	44.9	2867	2412	5379	2833
34	14.5	44.9	3036	2473	5508	3241
35	11.8	41.2	2894	2728	6022	3344
36	12.1	34.0	2372	1222	3594	2283
37	12.4	40.5	4143	2820	6063	3085
38	12.9	43.0	3537	2668	6205	3182
39	16.7	45.6	2578	2158	4735	2159
40	15.3	48.4	2604	2442	8046	2883
41	12.9	40.4	3429	2294	5723	3127
42	15.3	44.4	2390	2067	4656	2878
43	12.8	45.3	2170	1804	5383	2002
44	12.9	42.9	3478	2615	6091	3172

Table 15 Data on heart volume and physical working capacity and on pulse rate and blood pressures at rest in control group. HV = heart volume, PWC = physical working capacity BAP = brachial arterial pressure, BSA = body surface area. Blood pressure values are intra arterial and in mm.Hg

Case number	HV (ccm.)	HV (ccm./m^2 BSA)	PWC (kg.m./min.)	Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse
1	888	430	900	61	131	91	101	40
2	971	471	1060	89	117	80	83	37
3	716	420	900	64	139	84	101	55
4	790	439	1155	60	137	69	83	68
5	680	353	1128	51	128	63	89	65
6	703	399	828	84	172	111	125	61
7	968	464	762	68	128	72	88	56
8	860	473	900	76	136	76	100	60
9	689	394	1050	63	129	66	88	63
10	578	314	960	58	141	86	114	55
11	643	337	900	76	135	74	97	61
12	843	432	900	64	120	80	97	40
13	909	457	1200	64	137	80	110	57
14	660	333	900	75	142	93	108	49
15	771	428	810	60	152	80	112	72
16	953	467	1050	56	147	111	123	36
17	999	540	1200	50	117	63	81	54
18	725	41.	900	74	151	90	111	61
19	629	335	1010	72	145	83	99	62
20	791	452	842	69	141	85	100	56
21	695	353	720	73	123	71	87	52

Table 16. Data on heart volume and physical working capacity and on pulse rate and blood pressures at rest in hypotensive group. HV = heart volume, PWC = physical working capacity BAP = brachial arterial pressure, BSA = body surface. Blood pressure values are intra arterial and in mm.Hg

Case number	HV (ccm.)	HV (ccm./m^2 BSA)	PWC (kg.m./min.)	Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse
22	620	367	1112	63	100	63	80	37
23	655	395	950	50	105	74	89	31
24	521	294	900	69	107	71	84	36
25	714	379	800	52	106	67	81	39
26	478	288	900	64	123	67	84	56
27	735	435	900	57	107	67	74	40
28	773	447	900	61	110	78	89	32
29	660	357	1130	48	101	64	80	37
30	977	498	1200	63	107	71	84	36
31	812	456	835	68	112	71	83	41
32	761	369	1100	60	110	71	83	29
33	792	440	1020	60	108	64	74	45
34	773	455	1050	54	110	70	86	40
35	832	420	1050	57	110	80	89	30
36	470	298	510	73	126	63	84	63
37	748	394	1332	54	106	78	88	30
38	725	372	984	54	110	66	74	44
39	914	486	830	60	114	68	76	48
40	638	341	968	72	100	57	72	43
41	687	373	1050	54	143	72	95	71
42	541	311	700	54	80	57	67	33
43	741	372	830	54	105	79	89	26
44	810	422	1010	50	114	65	77	49

Table 17 Data on circulation times, blood flows, central blood volume, and pressure-flow relationships at rest in control subjects. CT = circulation time, CO = cardiac output, CI = cardiac index, SV = stroke volume, SI = stroke index, MCT = mean circulation time, CBV = central blood volume, PR = peripheral resistance, LVWI = left ventricular work index, LVSWI = left ventricular stroke work index, CE = central elasticity BSA = body surface area

Case number	CT (sec.)	CO (l./min.)	CI (l./min.)	SV (ml.)	SI (ml.)	MCT (sec.)	CBV (l)	CBV (l./m² BSA)	PR (dyn.sec. cm.⁻⁵)	LVWI (kg m./min./m² BSA)	LVSWI (gm.m./m² BSA)	CE (dyn./cm²/cm³)
1	10.8	5.60	2.75	83	45.0	20.1	1.89	0.92	1427	3.8	60.3	917
2	12.0	5.32	2.65	80	38.8	23.7	2.18	1.06	1349	3.4	43.1	711
3	10.0	5.11	2.83	79	48.1	17.0	1.45	0.85	1579	4.0	63.3	742
4	11.8	5.58	3.10	83	51.7	19.5	1.84	1.02	1189	3.5	58.4	456
5	11.6	6.63	3.52	130	68.8	24.8	2.75	1.45	1068	4.3	61.1	236
6	8.0	5.06	2.93	80	34.1	14.5	1.22	0.69	1975	5.0	58.0	1098
7	8.4	5.97	2.95	83	42.3	16.9	1.65	0.89	1170	4.4	50.8	784
8	11.3	4.34	2.35	57	31.3	22.5	1.87	1.03	1842	3.2	42.6	589
9	11.2	4.32	2.47	60	30.4	27.1	1.95	1.11	1501	2.8	44.1	762
10	11.8	7.16	3.89	123	66.8	26.8	3.17	1.72	1276	6.0	103.8	498
11	10.4	8.97	4.09	118	61.8	17.0	2.54	1.33	827	6.2	81.5	620
12	11.4	5.79	2.97	90	46.2	20.7	1.90	1.02	1339	3.9	60.9	513
13	10.8	5.02	2.62	88	44.3	20.3	1.80	0.95	1584	4.2	66.3	756
14	10.9	6.09	3.08	81	40.7	17.6	1.76	0.84	1410	4.5	59.8	387
15	10.8	7.02	3.80	117	65.0	21.7	2.84	1.41	1286	5.9	89.0	353
16	11.4	4.98	2.44	89	43.6	28.1	2.27	1.11	1974	4.1	72.9	717
17	12.0	4.25	2.29	85	44.9	23.7	1.67	0.90	1531	2.5	59.5	802
18	8.8	6.31	3.58	85	48.3	15.7	1.65	0.94	1408	5.6	72.9	735
19	10.4	9.07	4.82	128	67.0	18.9	2.86	1.52	873	6.5	90.2	294
20	9.5	6.53	3.51	95	56.6	19.3	2.19	1.25	1168	5.3	76.9	512
21	10.8	6.55	3.32	89	45.2	20.2	2.21	1.12	1063	3.9	52.5	334

Table 18. Data on circulation times, blood flows, central blood volume and pressure-flow relationships at rest in hypotensive subjects. CT = circulation time, CO = cardiac output, CI = cardiac index, SV = stroke volume, SI = stroke index, MCT = mean circulation time, CBV = central blood volume. PR = peripheral resistance, LVWI = left ventricular work index, LVSWI = left ventricular stroke work index, CE = central elasticity BSA = body surface area

Case number	CT (sec.)	CO (l./min.)	CI (l./m²)	SV (ml.)	SI (ml.)	MCT (sec.)	CBV (l)	CBV (l./m² BSA)	PR (dyn.sec. cm.⁻⁵)	LVWI (kg m./min./m² BSA)	LVSWI (gm.m./m² BSA)	CE (dyn./cm²/cm³)
22	11.2	6.57	3.29	68	52.1	25.8	2.39	1.42	1145	3.6	65.7	957
23	12.4	4.67	2.93	97	58.4	19.5	1.59	0.95	1496	3.5	60.7	609
24	12.4	5.09	2.88	74	41.8	20.8	1.75	0.99	1299	3.2	43.0	244
25	14.0	4.19	2.23	81	43.1	25.4	1.98	1.05	1548	2.5	47.5	707
26	11.2	5.83	3.57	83	56.0	19.2	1.89	1.17	1182	4.2	63.9	802
27	10.8	5.47	3.24	96	56.7	21.7	1.90	1.17	1081	3.3	57.1	[illegible]
28	12.8	6.26	3.61	102	59.0	23.3	2.43	1.40	1139	4.4	71.4	404
29	12.4	3.96	2.14	83	44.9	25.4	1.91	1.03	1615	2.3	43.9	540
30	10.8	6.34	3.54	110	54.2	20.1	2.32	1.18	930	4.9	64.1	458
31	10.0	3.60	2.08	56	31.5	18.6	1.14	0.64	1790	2.3	35.6	802
32	10.8	6.51	3.16	109	52.9	20.9	2.27	1.19	1619	3.9	59.7	[illegible]
33	12.0	6.40	3.61	108	60.0	22.4	2.42	1.34	811	3.6	60.4	474
34	14.0	4.32	2.64	80	47.1	25.4	2.04	1.20	1581	3.0	55.1	637
35	12.4	4.23	2.14	78	38.4	25.0	1.86	1.00	1682	2.8	44.5	640
36	11.4	4.08	2.52	84	34.4	18.6	1.82	0.84	1653	2.9	59.3	871
37	12.0	5.03	2.65	83	51.6	25.9	2.17	1.14	1298	3.1	61.8	251
38	10.2	4.61	2.38	85	43.6	25.7	2.74	1.41	1290	2.4	42.9	527
39	12.2	5.79	3.08	97	51.8	21.2	2.65	1.09	1049	3.2	52.3	580
40	10.0	6.25	3.34	87	46.8	19.2	2.60	1.07	922	3.3	45.5	203
41	12.0	4.25	2.38	83	47.8	24.7	2.34	1.27	1733	3.1	61.7	582
42	10.6	4.50	2.64	85	45.8	19.3	1.47	0.84	1167	2.4	44.5	536
43	11.0	4.30	2.18	88	40.2	20.1	1.45	0.73	1647	2.6	48.7	561
44	15.0	5.06	2.54	101	52.6	21.9	2.49	1.40	1221	2.7	55.1	341

Table 15. Data on heart volume and physical working capacity and on pulse rate and blood pressures at rest in control group. HV = heart volume, PWC = physical working capacity BAP = brachial arterial pressure, BSA = body surface area. Blood pressure values are intra-arterial and in mm.Hg

Case number	HV (ccm.)	HV (ccm./m² BSA)	PWC (kg.m./min.)	Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse
1	886	430	900	61	131	91	101	40
2	971	471	1060	69	117	80	93	37
3	716	420	900	64	139	84	101	55
4	790	439	1155	60	137	69	83	68
5	680	353	1128	51	128	63	89	65
6	703	399	828	84	172	111	125	61
7	966	464	762	68	128	72	88	56
8	860	473	900	76	136	76	100	60
9	689	394	1050	63	129	66	86	63
10	578	314	960	58	141	86	114	55
11	643	337	900	76	135	74	97	61
12	842	432	900	64	120	80	97	40
13	909	457	1200	64	137	80	110	57
14	680	333	900	75	142	93	108	49
15	771	428	810	60	152	80	112	72
16	953	467	1050	56	147	111	123	36
17	999	540	1200	50	117	63	81	54
18	725	412	900	74	151	90	111	61
19	629	335	1010	72	145	83	99	62
20	791	452	842	69	141	85	100	56
21	695	353	720	73	123	71	87	52

Table 16. Data on heart volume and physical working capacity and on pulse rate and blood pressures at rest in hypotensive group. HV = heart volume, PWC = physical working capacity BAP = brachial arterial pressure, BSA = body surface. Blood pressure values are intra-arterial and in mm.Hg

Case number	HV (ccm.)	HV (ccm./m² BSA)	PWC (kg.m./min.)	Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse
22	620	367	1112	63	100	63	80	37
23	655	395	950	50	105	4	89	31
24	521	294	900	69	107	71	84	36
25	714	379	900	52	106	67	81	39
26	478	288	900	64	123	67	84	56
27	735	435	900	57	107	67	74	40
28	773	447	900	61	110	78	89	32
29	660	357	1130	48	101	64	80	37
30	977	498	1200	63	107	71	84	36
31	812	456	935	66	112	71	83	41
32	761	369	1100	60	110	71	83	39
33	792	440	1020	60	109	64	74	45
34	773	455	1050	54	110	70	86	40
35	832	420	1050	57	110	80	89	30
36	470	299	510	75	126	63	84	63
37	748	394	1332	54	108	78	88	30
38	725	372	984	54	110	66	74	44
39	914	486	830	60	114	68	76	46
40	638	341	966	72	100	57	72	43
41	687	373	1050	54	143	72	95	71
42	541	311	700	54	90	57	67	33
43	741	372	830	54	105	79	89	28
44	810	422	1010	50	114	65	77	49

pressure, CT = circulation time, CO = cardiac output, CI = cardiac index, SV = stroke
WI = left ventricular work index, LVSWI = left ventricular stroke work index, CBV =

CI (L/min.)	SV (ml.)	SI (ml.)	MCT (sec.)	PR (dyn. sec. cm⁻⁵.)	LVWI (kg.m./min. /m².BSA)	LVSWI (gm.m./ m².BSA)	CBV (L)	CBV (L/m² BSA)
2.75	93	45.0	20.1	1427	3.8	60.8	1.89	0.92
5.82	102	49.5	13.2	818	9.7	82.7	2.80	1.36
+3.07	+ 9	+ 4.5	− 6.9	− 609	+ 5.9	+21.9	+0.91	+0.44
+112	+10	+10	−34	− 43	+155	+36	+48	+48
2.68	80	38.8	23.7	1349	3.4	49.1	2.18	1.06
5.73	119	57.3	15.5	784	9.0	90.3	3.05	1.48
+3.05	+39	+18.5	− 8.2	− 565	+ 5.6	+41.2	+0.87	+0.42
+114	+49	+49	−35	− 42	+165	+84	+40	+40
2.93	79	46.1	17.0	1579	4.0	63.3	1.45	0.85
9.92	143	83.6	11.2	529	15.1	127.3	3.17	1.85
+6.99	+64	+37.5	− 5.8	−1050	+11.1	+64.0	+1.72	+1.00
+222	+81	+81	−34	− 66	+278	+101	+119	+119
3.10	93	51.7	19.8	1189	3.5	58.4	1.84	1.02
5.55	111	61.7	12.9	851	6.9	76.3	2.14	1.19
+2.45	+18	+10	− 6.9	− 338	+ 3.4	+17.9	+0.30	+0.17
+79	+19	+19	−35	− 28	+97	+31	+16	+16
3.52	130	68.8	24.8	1068	4.3	61.1	2.73	1.45
5.76	104	55.0	21.2	—	—	—	3.82	2.03
+2.24	−26	−13.8	− 3.6	—	—	—	+1.09	+0.58
+64	−20	−20	−15	—	—	—	+39	+39
2.93	60	34.1	14.5	1975	5.0	58.0	1.22	0.69
4.34	64	36.4	10.6	—	—	—	1.35	0.77
+1.41	+ 4	+ 2.3	− 3.9	—	—	—	+0.13	+0.08
+51	+ 7	+ 7	−27	—	—	—	+11	+11
2.80	88	42.3	16.9	1178	4.4	50.6	1.85	0.89
5.00	88	42.3	13.6	—	—	—	2.40	1.15
+2.21	± 0	± 0	− 3.3	—	—	—	+0.55	+0.26
+77	± 0	± 0	−19	—	—	—	+29	+29
2.38	57	31.3	23.5	1842	3.2	42.6	1.87	1.03
7.04	112	61.5	13.7	—	—	—	2.93	1.41
+4.66	+55	+30.2	− 9.8	—	—	—	+1.06	+0.38
+196	+96	+96	−42	—	—	—	+57	+37
2.47	69	39.4	27.1	1591	2.8	46.1	1.95	1.11
7.96	113	64.6	12.5	—	—	—	2.90	1.66
+5.49	+44	+25.2	−14.6	—	—	—	+0.95	+0.55
+222	+64	+64	−54	—	—	—	+49	+49
3.89	123	66.8	26.6	1276	6.0	103.8	3.17	1.72
8.67	148	80.4	13.3	—	—	—	3.54	1.92
+4.78	+25	+13.6	−13.3	—	—	—	+0.37	+0.20
+123	+20	+20	−50	—	—	—	+12	+12
4.69	118	61.8	17.0	827	8.2	81.5	2.54	1.33
7.75	129	67.5	12.2	—	—	—	3.01	1.58
+3.06	+11	+ 5.7	− 4.8	—	—	—	+0.47	+0.25
+65	+ 9	+ 9	−28	—	—	—	+19	+19

Table 19 Effect of exercise on hemodynamics of control subjects. BAP = brachial arterial volume, SI = stroke index, MCT = mean circulation time, PR = peripheral resistance, LV central blood volume, BSA = body surface area
R = rest, E = exercise, Ch. = change, Ch% = change percentage

Case number		Pulse rate	BAP *syst.*	BAP *diast.*	BAP *mean*	BAP *pulse*	CT (sec.)	CO (L/min.)
1	R	61	131	91	101	40	10.8	5.66
	E	117	148	98	123	50	8.6	11.98
	Ch.	+ 56	+17	+ 7	+22	+10	—2.2	+ 6.32
	Ch%	+ 59	+13	+ 8	+22	+25	—20	+112
2	R	69	117	80	93	37	12.0	5.52
	E	99	149	89	116	60	8.4	11.81
	Ch.	+ 30	+32	+ 9	+23	+23	—3.6	+ 6.29
	Ch%	+ 43	+27	+11	+25	+62	—30	+114
3	R	64	139	84	101	55	10.0	5.11
	E	118	166	91	112	75	7.0	16.97
	Ch.	+ 54	+27	+ 7	+11	+20	—3.0	+11.86
	Ch%	+ 84	+19	+ 8	+11	+36	—30	+232
4	R	60	137	69	83	68	11.0	5.58
	E	90	151	74	91	77	8.0	9.99
	Ch.	+ 30	+14	+ 5	+ 8	+ 9	—3.0	+ 4.41
	Ch%	+ 50	+10	+ 7	+10	+13	—27	+79
5	R	51	128	63	89	65	11.6	6.65
	E	105	—	—	—	—	9.2	10.88
	Ch.	+ 54	—	—	—	—	—2.4	+ 4.23
	Ch%	+106	—	—	—	—	—21	+64
6	R	84	172*)	111	125	61	8.0	5.06
	E	120	—	—	—	—	6.0	7.64
	Ch.	+ 36	—	—	—	—	—2.0	+ 2.58
	Ch%	+ 43	—	—	—	—	—25	+51
7	R	68	128	72	88	56	8.4	5.97
	E	120	—	—	—	—	8.0	10.59
	Ch.	+ 52	—	—	—	—	—0.4	+ 4.62
	Ch%	+ 76	—	—	—	—	—5	+77
8	R	76	136	76	100	60	11.2	4.34
	E	114	—	—	—	—	8.0	12.82
	Ch.	+ 38	—	—	—	—	—3.2	+ 8.48
	Ch%	+ 50	—	—	—	—	—29	+193
9	R	63	129	66	86	63	11.2	4.32
	E	123	—	—	—	—	6.8	13.93
	Ch.	+ 60	—	—	—	—	—4.4	+ 9.61
	Ch%	+ 95	—	—	—	—	—39	+222
10	R	58	141	86	114	55	11.8	7.16
	E	108	—	—	—	—	7.2	15.95
	Ch.	+ 50	—	—	—	—	—3.6	+ 8.79
	Ch%	+ 86	—	—	—	—	—31	+126
11	R	76	135	74	97	61	10.4	8.97
	E	114	—	—	—	—	7.2	14.81
	Ch.	+ 38	—	—	—	—	—3.2	+ 5.84
	Ch%	+ 50	—	—	—	—	—31	+65

pressure, CT = circulation time, CO = cardiac output, CI = cardiac index, SV = stroke
WI = left ventricular work index, LVSWI = left ventricular strok work index, CBV =

CI (L/min.)	SV (ml.)	SI (ml.)	MCT (sec.)	PR (dyn. sec. cm⁻⁵)	LVWI (kg.m./min. /m²BSA)	LVSWI (gm.m./ m²BSA)	CBV (L)	CBV (L/m² BSA)
2.75	93	45.0	20.1	1427	3.8	60.8	1.89	0.92
5.82	102	49.5	13.2	818	9.7	82.7	2.80	1.36
+3.07	+ 9	+ 4.5	− 6.9	− 609	+ 5.9	+21.9	+0.91	+0.44
+112	+10	+10	−34	− 43	+155	+36	+48	+48
2.68	80	38.8	23.7	1349	3.4	49.1	2.18	1.06
5.73	119	57.3	15.5	784	9.0	90.3	3.05	1.48
+3.05	+39	+18.5	− 8.2	− 565	+ 5.6	+41.2	+0.87	+0.42
+114	+49	+49	−35	− 42	+165	+84	+40	+40
2.83	79	46.1	17.0	1579	4.0	63.3	1.45	0.85
8.83	143	83.6	11.2	529	15.1	127.3	3.17	1.85
+6.00	+64	+37.5	− 5.8	−1050	+11.1	+64.0	+1.72	+1.00
+212	+81	+81	−34	− 66	+278	+101	+119	+119
3.10	93	51.7	19.8	1189	3.5	58.4	1.84	1.02
5.55	111	61.7	12.9	851	6.9	76.3	2.14	1.19
+2.45	+18	+10	− 6.9	− 338	+ 3.4	+17.9	+0.30	+0.17
+79	+19	+19	−35	− 28	+97	+31	+16	+16
3.52	130	68.8	24.6	1068	4.3	81.1	2.75	1.45
5.76	104	55.0	21.0	—	—	—	3.84	2.03
+2.24	−26	−13.8	− 3.6	—	—	—	+1.09	+0.58
+64	−20	−20	−15	—	—	—	+39	+39
2.93	60	34.1	14.5	1075	5.0	85.0	1.22	0.69
4.34	64	36.4	10.6	—	—	—	1.35	0.77
+1.41	+ 4	+ 2.3	− 3.9	—	—	—	+0.13	+0.08
+48	+ 7	+ 7	−27	—	—	—	+11	+12
2.86	85	42.3	16.9	1178	4.4	50.6	1.85	0.98
5.09	85	42.3	13.6	—	—	—	2.40	1.15
+2.23	± 0	± 0	− 3.3	—	—	—	+0.55	+0.26
+77	± 0	± 0	−19	—	—	—	+29	+29
2.36	67	31.3	23.5	1942	3.2	42.6	1.87	1.03
7.04	112	61.5	13.7	—	—	—	2.93	1.41
+4.68	+45	+30.2	− 9.8	—	—	—	+1.06	+0.38
+193	+66	+96	−42	—	—	—	+57	+37
2.47	69	38.4	27.1	1591	2.8	46.1	1.95	1.11
7.96	113	64.6	12.5	—	—	—	2.90	1.66
+5.49	+44	+25.2	−14.6	—	—	—	+0.95	+0.55
+222	+64	+64	−54	—	—	—	+49	+49
3.89	123	68.8	26.6	1278	6.0	103.8	3.17	1.72
8.67	148	80.4	13.3	—	—	—	3.54	1.92
+4.78	+25	+13.6	−13.3	—	—	—	+0.37	+0.20
+126	+20	+20	−50	—	—	—	+12	+12
4.00	118	61.8	17.0	887	6.2	81.5	2.54	1.33
7.35	129	67.5	12.2	—	—	—	3.01	1.58
+3.06	+11	+ 5.7	− 4.8	—	—	—	+0.47	+0.25
+43	+ 9	+ 9	−28	—	—	—	+19	+19

Case number		Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse	CT (sec.)	CO (L/min.)
12	R	64	120	80	97	40	11.4	5.79
	E	124	—	—	—	—	6.8	12.68
	Ch.	+ 60	—	—	—	—	—4.6	+ 6.89
	Ch%	+ 94	—	—	—	—	—40	+119
13	R	64	137	80	100	57	10.8	5.62
	E	106	151	89	118	62	7.8	10.91
	Ch.	+ 42	+14	+ 9	+ 8	+ 5	—3.0	+ 5.29
	Ch%	+ 66	+10	+11	+ 7	+ 8	—28	+94
14	R	75	142	93	108	49	10.0	6.09
	E	104	—	—	—	—	8.0	15.17
	Ch.	+ 29	—	—	—	—	—2.0	+ 9.08
	Ch%	+ 39	—	—	—	—	—20	+149
15	R	60	152	80	11.	72	10.6	7.02
	E	116	178	87	123	91	6.2	14.29
	Ch.	+ 56	+26	+ 7	+11	+19	—4.6	+ 7.27
	Ch%	+ 93	+17	+ 9	+10	+26	—43	+105
16	R	56	147	111	123	36	13.4	4.96
	E	108	—	—	—	—	7.6	13.94
	Ch.	+ 52	—	—	—	—	—5.8	+ 8.96
	Ch%	+ 93	—	—	—	—	—43	+179
17	R	50	117	63	81	54	12.0	4.23
	E	104	—	—	—	—	8.0	8.14
	Ch.	+ 54	—	—	—	—	—4.0	+ 3.91
	Ch%	+108	—	—	—	—	—33	+92
18	R	74	151	90	111	61	8.8	6.31
	E	111	—	—	—	—	7.0	9.25
	Ch.	+ 37	—	—	—	—	—1.8	+ 2.94
	Ch%	+ 50	—	—	—	—	—20	+47
19	R	72	145	83	99	62	10 4	9.07
	E	114	169	90	120	79	8.0	—
	Ch.	+ 42	+24	+ 7	+21	+17	—2.4	—
	Ch%	+ 58	+17	+ 8	+21	+28	—23	—
20	R	69	141	85	100	56	9.8	6.83
	E	117	—	—	—	—	7.2	14.13
	Ch.	+ 48	—	—	—	—	—2.6	+ 7.30
	Ch%	+ 69	—	—	—	—	—26	+107
21	R	73	123	71	87	52	10.8	6.55
	E	120	151	80	94	71	7.6	14 01
	Ch.	+ 47	+28	+ 9	+ 7	+19	—3.2	+ 7.46
	Ch%	+ 64	+23	+13	+ 8	+37	—29	+114

) Re-examined after two months BAP at rest 150/91

CI (L/min.)	SV (mL)	SI (mL)	MCT (sec.)	PR (dyn. sec. cm^{-5})	LVWI (kg.m./min. /m BSA)	LVSWI (gm.m./ m^2 BSA)	CBV (L)	CBV (L/m^2 BSA)
2.97	90	48.2	20.7	1339	3.9	60.9	1.99	1.02
6.50	102	52.3	12.4	—	—	—	2.62	1.34
+3.53	+12	+4.1	−8.3	—	—	—	+0.63	+0.32
+119	+13	+13	−40	—	—	—	+32	+32
2.82	88	44.3	20.3	1564	4.2	66.3	1.90	0.95
5.42	102	51.3	13.6	864	8.8	72.3	2.47	1.24
+2.60	+14	+7.0	−6.7	−700	+4.6	+6.0	+0.57	+0.29
+94	+16	+16	−33	−45	+109	+9	+30	+30
3.08	81	40.7	17.6	1410	4.5	50.8	1.76	0.84
7.66	137	69.2	10.1	—	—	—	2.55	1.29
+4.58	+56	+28.5	−7.5	—	—	—	+0.79	+0.45
+149	+69	+69	−43	—	—	—	+45	+45
3.80	117	65.0	21.7	1266	5.9	99.0	2.54	1.41
7.80	124	68.9	13.9	683	13.4	115.2	3.33	1.85
+4.00	+7	+3.9	−7.8	−583	+7.5	+16.2	+0.79	+0.44
+105	+6	+6	−36	−46	+127	+16	+31	+31
2.44	89	43.6	28.1	1974	4.1	72.9	2.27	1.11
6.80	129	63.2	14.2	—	—	—	3.29	1.61
+4.36	+40	+19.6	−13.9	—	—	—	+1.02	+0.50
+179	+45	+45	−49	—	—	—	+45	+45
2.29	85	44.9	23.7	1531	2.5	60.5	1.67	0.90
4.40	78	42.2	16.1	—	—	—	2.18	1.18
+2.11	−7	−2.7	−7.6	—	—	—	+0.51	+0.28
+92	−8	−6	−32	—	—	—	+31	+31
3.58	85	48.3	15.7	1408	5.4	72.9	1.65	0.94
5.26	83	47.2	10.4	—	—	—	1.60	0.91
+1.68	−2	−1.1	−5.3	—	—	—	−0.05	−0.03
+47	−2	−2	−34	—	—	—	−3	−3
4.82	126	67.0	18.9	673	6.5	90.2	2.86	1.52
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
3.91	99	55.6	19.3	1100	5.3	76.9	2.19	1.25
8.07	121	68.1	13.0	—	—	—	3.06	1.75
+4.16	+22	+12.5	−6.3	—	—	—	+0.87	+0.50
+107	+22	+22	−33	—	—	—	+39	+39
3.32	89	45.2	20.2	1063	3.9	53.5	2.21	1.12
7.11	117	59.4	14.0	544	9.1	75.9	3.27	1.66
+3.79	+28	+14.2	−6.2	−519	+5.2	+22.4	+1.06	+0.54
+114	+31	+31	−31	−49	+133	+42	+48	+48

Table 20. Effect of exercise on hemodynamics of hypotensive subjects, BAP = brachial stroke volume, SI = stroke index, MCT = mean circulation time, PR = peripheral resistance, central blood volume, BSA = body surface area
R = rest, E = exercise, Ch. = change, Ch% = change percentage

Case number		Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse	CT (sec.)	CO (L/min.)
22	R	63	100	63	80	37	11.2	5.57
	E	108	133	66	89	67	7.6	9.48
	Ch.	+ 45	+33	+ 3	+ 9	+ 30	—3.6	+ 3.91
	Ch%	+ 71	+33	+ 5	+11	+ 81	—32	+ 71
23	R	50	105	74	89	31	12.4	4.87
	E	108	—	—	—	—	8.0	14.74
	Ch.	+ 58	—	—	—	—	—4.4	+ 9.87
	Ch%	+116	—	—	—	—	—35	+203
24	R	69	107	71	84	36	12.4	5.09
	E	115	167	78	102	89	7.8	12.41
	Ch.	+ 46	+60	+ 7	+18	+ 53	—4.6	+ 7.32
	Ch%	+ 67	+56	+10	+21	+147	—37	+144
25	R	52	106	67	81	39	14.0	4.19
	E	98	130	85	109	45	9.2	9.48
	Ch.	+ 46	+24	+18	+28	+ 6	—4.8	+ 5.29
	Ch%	+ 88	+23	+27	+35	+ 15	—34	+126
26	R	64	123	67	84	56	11.2	5.93
	E	108	146	82	101	64	7.2	11.76
	Ch.	+ 44	+23	+15	+17	+ 8	—3.0	+ 5.83
	Ch%	+ 69	+19	+22	+20	+ 14	—27	+ 88
27	R	57	107	67	74	40	10.6	5.47
	E	108	130	80	105	50	6.6	11.15
	Ch.	+ 51	+23	+13	+31	+ 10	—4.2	+ 5.68
	Ch%	+ 89	+22	+19	+42	+ 25	—39	+104
28	R	61	110	78	89	32	12.8	6.25
	E	108	—	—	—	—	8.4	15.45
	Ch.	+ 47	—	—	—	—	—4.4	+ 9.20
	Ch%	+ 77	—	—	—	—	—34	+147
29	R	48	101	64	80	37	12.4	3.96
	E	102	136	83	111	53	8.2	9.38
	Ch.	+ 54	+35	+19	+31	+ 16	—4.2	+ 5.42
	Ch%	+113	+35	+29	+39	+ 43	—34	+132
30	R	63	107	71	84	36	10.8	6.94
	E	93	118	77	94	41	8.2	11.05
	Ch.	+ 30	+11	+ 6	+10	+ 5	—2.6	+ 4.11
	Ch%	+ 48	+10	+ 8	+12	+ 14	—24	+ 59
31	R	66	112	71	83	41	10.0	3.69
	E	108	128	94	113	34	6.8	9.14
	Ch.	+ 42	+16	+23	+30	— 7	—3.2	+ 5.45
	Ch%	+ 64	+14	+32	+36	— 17	—32	+148
32	R	60	110	71	83	39	10.8	6.51
	E	102	143	83	103	60	6.8	14.22
	Ch.	+ 42	+33	+12	+20	+ 21	—4.0	+ 7.71
	Ch%	+ 70	+30	+17	+24	+ 54	—37	+118
33	R	60	109	64	74	45	12.0	6.49
	E	104	156	85	112	71	7.4	13.76
	Ch.	+ 44	+47	+21	+38	+ 26	—4.6	+ 7.27
	Ch%	+ 73	+43	+33	+51	+ 58	—38	+112

arterial pressure, CT = circulation time, CO = cardiac output, CI = cardiac index, SV =
LVWI = left ventricular work index, LVSWI= left ventricular stroke work index, CBV =

CI (L/min.)	SV (mL)	SI (mL)	MCT (sec.)	PR (dyn. sec. cm^{-5})	LVWI (kg.m./min. /m^2BSA)	LVSWI (gm.m./ m BSA)	CBV (L)	CBV (L/m^2 BSA)
3.29	[illegible]	52.1	25.8	1145	3.6	58.7	2.39	1.42
5.61	88	52.1	15.2	780	6.8	63.1	2.40	1.42
+ 2.32	± 0	± 0	—10.6	—365	+ 3.2	+ 4.4	+0.01	± 0
+ 71	± 0	± 0	—41	—33	+ 89	+11	± 0	± 0
2.83	97	58.4	19.5	1496	3.5	60.7	1.58	0.95
8.68	136	81.9	12.6	—	—	—	3.09	1.86
+ 5.85	+39	+23.5	— 6.9	—	—	—	+1.51	+0.91
+203	+40	+40	—35	—	—	—	+96	+96
2.88	74	41.8	20.6	1299	3.2	45.0	1.75	0.99
7.01	108	61.0	12.4	659	9.7	84.6	2.56	1.45
+ 4.13	+34	+19.2	— 8.2	—640	+ 6.5	+39.6	+0.81	+0.46
+144	+46	+46	—40	— 49	+203	+88	+46	+46
2.23	81	43.1	28.4	1546	2.5	47.5	1.98	1.05
5.04	97	51.6	17.1	919	7.5	76.5	2.70	1.44
+ 2.81	+16	+ 8.5	—11.3	—627	+ 5.0	+29.0	+0.72	+0.39
+126	+19	+19	—40	— 41	+200	+61	+36	+36
3.57	[illegible]	56.0	19.2	1132	4.2	63.9	1.89	1.17
7.08	109	65.7	13.1	686	9.7	80.2	2.57	1.55
+ 3.51	+16	+ 9.7	— 6.1	—446	+ 5.5	+16.3	+0.68	+0.38
+ 98	+17	+17	—32	— 39	+131	+26	+36	+36
3.24	96	56.7	21.7	1061	3.3	57.1	1.98	1.17
6.59	103	60.9	13.5	732	9.4	86.9	2.51	1.48
+ 3.35	+ 7	+ 4.2	— 8.2	—329	+ 6.1	+29.8	+0.53	+0.31
+104	+ 7	+ 7	—38	— 30	+185	+52	+27	+27
3.61	102	59.0	23.3	1130	4.4	71.4	2.43	1.40
8.83	143	82.7	14.1	—	—	—	3.63	2.09
+ 5.22	+41	+23.7	— 9.2	—	—	—	+1.20	+0.69
+147	+40	+40	—39	—	—	—	+49	+49
2.14	83	44.9	26.4	1615	2.3	48.9	1.91	1.03
5.07	92	49.7	14.3	948	7.7	75.0	2.24	1.21
+ 2.93	+ 9	+ 4.8	—12.1	—667	+ 5.4	+26.1	+0.33	+0.18
+137	+11	+11	—46	— 41	+235	+53	+17	+17
3.54	110	58.1	20.1	930	4.0	64.1	2.32	1.18
5.64	119	62.7	9.6	680	7.5	77.6	1.77	0.90
+ 2.10	+ 9	+ 4.6	—10.5	—250	+ 3.5	+13.5	—0.55	—0.28
+ 59	+ 8	+ 8	— 52	— 27	+ 88	+21	—24	—24
2.08	56	31.5	18.6	1790	2.3	35.6	1.14	0.64
5.13	85	47.7	11.6	980	7.9	73.3	1.77	0.99
+ 3.05	+29	+16.2	— 7.0	—808	+ 5.6	+37.7	+0.63	+0.35
+146	+53	+53	—38	— 45	+243	+106	+55	+55
3.16	109	52.9	20.9	1019	3.6	59.7	2.27	1.10
6.90	139	67.5	11.8	572	9.7	94.5	2.79	1.35
+ 3.74	+30	+14.6	— 9.1	—447	+ 6.1	+34.8	+0.52	+0.25
+118	+28	+28	—44	— 44	+169	+58	+23	+23
3.61	108	60.0	22.4	911	3.6	60.4	2.42	1.34
7.64	132	73.3	14.1	631	11.6	111.5	3.23	1.79
+ 4.03	+24	+13.3	— 8.3	—280	+ 8.0	+51.1	+0.81	+0.45
+112	+22	+22	—37	— 30	+222	+85	+33	+33

Case number		Pulse rate	BAP syst.	BAP diast.	BAP mean	BAP pulse	CT (sec.)	CO (l./min.)
34	R	54	110	70	86	40	14.0	4.32
	E	112	139	78	98	63	8.8	—
	Ch.	+ 58	+29	+ 8	+12	+ 23	—5.2	—
	Ch%	+108	+26	+ 9	+14	+ 58	—37	—
35	R	57	110	80	89	30	12.4	4.23
	E	105	—	—	—	—	7.6	8.35
	Ch.	+ 48	—	—	—	—	—4.8	+ 4.12
	Ch%	+ 84	—	—	—	—	—39	+ 97
36	R	75	126	63	84	63	11.4	4.06
	E	138	151	61	90	90	7.0	9.31
	Ch.	+ 63	+25	— 2	+ 6	+ 27	—4.4	+ 5.25
	Ch%	+ 84	+19	— 3	+ 7	+ 43	—39	+129
37	R	54	108	78	88	30	13.0	5.03
	E	96	—	—	—	—	9.0	10.66
	Ch.	+ 42	—	—	—	—	—4.0	+ 5.63
	Ch%	+ 78	—	—	—	—	—31	+112
38	R	54	110	68	74	44	16.2	4.61
	E	105	—	—	—	—	11.6	10.77
	Ch.	+ 51	—	—	—	—	—4.6	+ 6.16
	Ch%	+ 94	—	—	—	—	—28	+134
39	R	60	114	68	76	46	12.2	5.79
	E	111	—	—	—	—	7.2	9.22
	Ch.	+ 51	—	—	—	—	—5.0	+ 3.43
	Ch%	+ 85	—	—	—	—	—41	+ 59
40	R	72	100	57	72	43	10.0	6.25
	E	108	—	—	—	—	9.0	14.52
	Ch.	+ 36	—	—	—	—	—1.0	+ 8.27
	Ch%	+ 50	—	—	—	—	—10	+132
41	R	54	143	72	95	71	12.0	4.38
	E	90	—	—	—	—	6.0	10.68
	Ch.	+ 36	—	—	—	—	—6.0	+ 6.30
	Ch%	+ 67	—	—	—	—	—50	+144
42	R	54	90	57	67	33	10.6	4.59
	E	120	—	—	—	—	8.8	9.78
	Ch.	+ 66	—	—	—	—	—1.8	+ 5.19
	Ch%	+122	—	—	—	—	—17	+113
43	R	54	105	79	89	26	11.0	4.33
	E	120	—	—	—	—	6.2	10.22
	Ch.	+ 66	—	—	—	—	—4.8	+ 5.89
	Ch%	+122	—	—	—	—	—44	+138
44	R	50	114	65	77	49	15.0	5.08
	E	92	—	—	—	—	9.6	12.74
	Ch.	+ 42	—	—	—	—	—5.4	+ 7.66
	Ch%	+ 84	—	—	—	—	—36	+151

CI (l/min)	SV (ml.)	SI (ml.)	MCT (sec.)	PR (dyn. sec. cm⁻⁵)	LVWI (kg.m./min. /m. BSA)	LVSWI (gm.m./ m² BSA)	CBV (L)	CBV (L/m² BSA)
2.54	60	47.1	28.4	1591	3.0	53.1	2.04	1.20
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
2.14	76	38.4	25.8	1082	2.6	48.5	1.98	1.00
4.21	80	40.4	17.4	—	—	—	2.41	1.22
+ 2.07	+ 4	+ 2.0	— 8.4	—	—	—	+0.43	+0.22
+ 97	+ 5	+ 5	—32	—	—	—	+22	+22
2.52	54	34.4	19.5	1653	2.9	39.3	1.32	0.84
5.93	69	43.9	11.9	773	7.3	53.7	1.85	1.18
+ 3.41	+15	+ 9.5	— 7.6	—880	+ 4.4	+14.4	+0.53	+0.34
+129	+28	+28	—39	— 53	+152	+37	+40	+40
2.63	93	51.6	25.9	1398	3.1	61.8	2.17	1.14
5.61	111	58.4	17.9	—	—	—	3.18	1.67
+ 2.98	+18	+ 6.8	— 8.0	—	—	—	+1.01	+0.53
+113	+19	+13	—31	—	—	—	+47	+47
2.36	85	42.6	35.7	1280	2.4	43.9	2.74	1.41
5.52	103	51.8	22.1	—	—	—	3.18	1.67
+ 3.16	+18	+ 9.2	—13.6	—	—	—	+0.44	+0.26
+134	+21	+21	—38	—	—	—	+16	+16
3.08	97	51.8	21.2	1049	3.2	63.2	2.05	1.09
4.90	83	44.1	13.8	—	—	—	2.12	1.13
+ 1.82	—14	— 7.5	— 7.4	—	—	—	+0.07	+0.04
+ 59	—14	—14	—35	—	—	—	+ 3	+ 3
3.34	87	46.5	19.2	922	3.3	48.5	2.00	1.07
7.76	134	71.7	10.9	—	—	—	2.64	1.41
+ 4.42	+47	+25.2	— 8.3	—	—	—	+0.64	+0.34
+132	+54	+54	—43	—	—	—	+32	+32
2.38	88	47.8	34.7	1733	3.1	61.7	2.34	1.27
5.80	119	64.7	18.9	—	—	—	3.36	1.83
+ 3.42	+31	+16.9	—15.8	—	—	—	+1.02	+0.56
+144	+35	+35	—46	—	—	—	+44	+44
2.64	85	48.8	19.2	1167	2.4	44.5	1.47	0.84
5.62	82	47.1	12.6	—	—	—	2.02	1.16
+ 2.98	— 3	— 1.7	— 6.6	—	—	—	+0.55	+0.32
+113	— 4	— 4	—34	—	—	—	+37	+37
2.18	80	40.2	20.1	1547	2.8	48.7	1.45	0.73
5.14	85	42.7	10.6	—	—	—	1.81	0.91
+ 2.96	+ 5	+ 2.5	— 9.5	—	—	—	+0.36	+0.18
+135	+ 6	+ 6	—47	—	—	—	+25	+25
2.54	101	52.6	31.9	1221	2.7	53.1	2.69	1.40
6.63	138	71.9	18.2	—	—	—	3.86	2.01
+ 4.09	+37	+19.3	—13.7	—	—	—	+1.17	+0.61
+151	+37	+37	—43	—	—	—	+43	+43

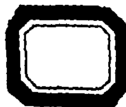
ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 379

HAEMOPHILIA IN SWEDEN

PART I

PART II



Accompanies volume 171

Stockholm 1962

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FROM THE CHEMISTRY DEPARTMENT II (HEAD ERIK JORPES, M.D.), KAROLINSKA INSTITUTET STOCKHOLM, THE DEPARTMENT OF MEDICINE I (HEAD E. SKÖLD M.D.), S T ERIKS SJUKHUS, STOCKHOLM, AND THE DEPARTMENT OF MEDICINE (HEAD J WALDENSTRÖM), UNIVERSITY OF LUND ALLMÄNNA SJUKHUSET MALMÖ SWEDEN

HAEMOPHILIA IN SWEDEN

ACTA MEDICA SCANDINAVICA
SUPPLEMENTUM 379

STOCKHOLM 1962

These investigations have been supported by grants from the Swedish Medical Research Council Harald och Greta Jeanssons Stiftelse, Stiftelsen Gustaf och Tyra Svenssons Minne Stiftelsen Therese och Johan Anderssons Minne, Eva och Oscar Ahréns Stiftelse Karolinska Institutet, Research Grants No H-4295 (R 1 C 1 and C 2) from the National Heart Institute National Institutes of Health U.S Public Health Service Syskonen Wessléns Stiftelse and Knut och Alice Wallenbergs Stiftelse Stockholm.

STOCKHOLM
K L BECKMANS TRYCKERIER AB
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1962

PART I

PART I

Acta Medica Scandinavica, Supplementum 379 1962

From Chemistry Department II (Head Erik Jorpes, M.D.), Karolinska Institutet Stockholm, Sweden

A PILOT PLANT

For the preparation of a human plasma fraction containing the human antihaemophilic factor A (factor VIII) and v Willebrand' factor

By

J Erik Jorpes Birger Blombäck, Margareta Blombäck and Staffan Magnusson

A Fibrinogen Preparation Containing Factor VIII

In continuation of the earlier work on heparin performed at the Chemistry Department II of Karolinska Institut t Stockholm (12, 14) a study of some coagulation factors was taken up in 1954 (13) Work on fibrinolysis was started by Per Wallén Birger and Margareta Blombäck took up a tudy of the heparin co-factor of plasma. Since *fibrinogen* the most suitable substrate for the titration of blood coagulation factors, was not easily available at that time, B and M Blombäck concentrated their efforts on the preparation of fibrinogen. They succeeded in elaborating a new technique (1) which gave a very good *material.*

The starting material Cohn s fraction I, which consists of up to 50 per cent fibrinogen, could be purified by repeated extractions of the precipitate with a one molar glycine solution in a citrate buffer ionic strength 0.3 pH 6.8, and containing a suitable alcohol concentration, i.e. 0.5 per cent. Lipoproteins, prothrombin and other contaminating proteins went into solution. Fibrinogen remained insoluble Coagulability of about 88 per cent was achieved, with a yield of 95 per cent of the fibrinogen present in fraction I

Supported by grants from the Swedish Medical Research Council, Harald och Greta Jea soms Stiftelse Stiftelsen Gustaf och Tyra Svenssons Mi ne Karolinska Institutet, Research Grants No H-4235 (H 1 C 1 and C 2) from the National Heart Institute, National Institutes of Health, U. S. Public Health Service, Syskonen Wessléns Stiftelse and Knut och Alice Wallenbergs Stiftelse, Stockholm.

The principle underlying this purification procedure is the property of glycine to increase the solubility of amino acids and peptides, as demonstrated already by S P L Sörensen and his school Glycine with its small molecular volume, is highly suitable for penetration into the network of the large protein precipitates, where the zwitterion decreases the protein protein interaction which holds the different particles together This principle was adopted by Cohn *et al* in their method 10 of 1950 for the separation of γ-globulin from β_1 lipoprotein in a human plasma precipitate. However if present in high concentration e.g a one molar solution glycine reduces the solubility of fibrinogen at the same time as it enhances the solubility of the contaminating plasma proteins

The preparation called fraction I—0 proved to be stable in sterile solution even at room temperature Through further fractionation 98—100 per cent purity as measured by the coagulability of the product could be achieved The yield of the final product was 40—50 per cent of the fibrinogen in fraction I for human material and 55—60 per cent for bovine material

The freeze-dried sterile fibrinogen preparation with 88 per cent coagulability was far more suitable for intravenous use in man than the more or less unstable fibrinogen preparations previously available

The preparations with 98—100 per cent purity also formed a particularly suitable material for the studies of the chemical changes taking place during the transformation of fibrinogen to fibrin later performed by B Blomback and co-workers (2—8)

The antihaemophilic factor A (factor VIII) known to be precipitated together with the fibrinogen in Cohn s fraction I was not removed when the precipitate was extracted twice with the one molar glycine solution It was in fact, recovered in the fibrinogen precipitate of 88 per cent coagulability (fraction I—0) from both human and bovine plasma, the yield being sometimes up to 100 per cent The concentration of AHF in fraction I—0 was 25—50 times that in the plasma calculated on a protein basis AHF could be separated from the bulk of fibrinogen in fraction I—0 by precipitating at an ethanol concentration of 0.5 per cent glycine 0 3 M ionic strength 0 1 and temperature O C (Fraction I—1A) (10 11)

Fraction I—0 proved to be suitable for the treatment of haemophilia A and did not give any side reactions

New and completely unexpected results were obtained when it was found that the preparation could be used for treatment of acute bleeding episodes in patients suffering from von Willebrand's disease as well (15 16 17) It did not only correct the AHF deficiency but also normalized the prolonged bleeding time in these patients

Because the AHF was to a large extent adsorbed during sterile filtration separation of the plasma Cohn fractionation extraction of fraction I with the glycine solution and subsequent freeze-drying, has been performed under aseptic conditions

In the early 1950 s, a unit for Cohn fractionation of plasma proteins as constructed by B Månsson and O

Ramgren and home-made freeze-drying equipment were installed at the Chemistry Department II at a total cost of about 25 000 Sw.crs.¹) This made it possible to start fractionation experiments under aseptic conditions.

Fraction I prepared according to Cohn's method 6 had been used by several authors, but as late as 1955 Winterstein of Basle, Koller of Zürich, and Deutsch of Vienna had expressed the view that the commercially available preparations were even less active than fresh human plasma. Solutions of fraction I—O had an AHF activity of 4—8 times that of fresh plasma, on the contrary, and could be given without any side reactions, and with a continued favourable effect after repeated administration to one and the same patient. The freeze-dried product could be stored at —20°C for at least 18 months without loss of activity. The use of fraction I—0 in the treatment of haemophilia A had, therefore to be seriously considered.

When, in May 1957, 9 patients had been treated successfully in connexion with major or minor operations, some of them repeatedly, the Federation of the Swedish County Council (Landstingsförbundet) was informed about the new possibility of treating haemophiliacs. The suggestion was made that our group at Karolinska Institutet should take up, in connexion with our research work, small-scale production of the AHF-containing fibrinogen, fraction I—0 provided that the costs would be covered.

The Federation reacted favourably to this suggestion by authorizing doctors in charge of hospital unit to order fraction I—0 at the expense of the hospital in the same way that blood or plasma is obtained from the Blood Transfusion Centres. The most serious hindrance to the introduction of specific therapy with the concentrated AHF preparation was thereby eliminated, and steps could be taken for further developing the fractionation operations.

Grants from Stiftelsen Gustaf och Tyra Svenssons Minne and the Swedish State Medical Research Council enabled us during the period 1957—1959 to replace the old plasma fractionation unit with a new one, including a temperature-regulated fractionation bath with 6 single cells (Fig. 14) (AB Kylteknik Huddinge, Sweden) a large refrigerated centrifuge, SR 3 (International Equipment Co., Boston) and additional new freeze-drying equipment with a capacity of 5 litres of water per load (AB Kylteknik) at a total cost of 123,000 Sw.crs. Blood was obtained from the Blood Transfusion Centre of the City of Stockholm at Sabbatsbergs Sjukhus (Dr Olof Ramgren)

The fractionation work was initially carried out by B. and M. Blombäck personally and later under their supervision. The details of the fractionation procedure as given by them (1) were supplemented in 1960 (9)

A New Fractionation Laboratory

In 1958, M Blombäck and I. M. Nilsson (18) reported on the first 12 patients with severe *classical haemophilia A* treated with the concentrated AHF preparation. Two years earlier the efficiency of the preparation had been proved in the most dramatic

¹) 1 U.S. dollar = approx. 5 Sw.crs.

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In the early 1950 s, *a unit for Cohn fractionation of plasma proteins* as constructed by B Månsson and O

Birger Blombäck was appointed on May 1 1961

Preparation of Fraction I—O

Since a good yield of AHF cannot be obtained after sterile filtration (through sintered glass) of fraction I—O the whole fractionation procedure has been performed in a closed system under aseptic conditions.

Blood collection In order to minimize the risk of contamination with hepatitis virus, initially plasma from only 8 donors was pooled each time (175—225 ml of non-lipoemic plasma from each donor) all the donors belonging either to the same ABO blood group as the patient or to the O group. Later 10 16 or 30 donations were used for each batch, and no attention was paid to the blood group of the patients, but the donors for each particular batch belonged to the same ABO group.

A small sample of blood is drawn into a test tube at the end of blood letting If from the colour of the plasma suspicion of liv r disease is aroused, the plasma in question is not included in the pool The donors are instru ted to report immediately to the Blood Tran fusi n Centre on appearance of any signs and symptoms compatible with ea ly hepatitis.

400—440 ml of blood are drawn in the course of about 6 minutes, with careful manual stirring, into illconized centrifuge bottles) (fitting the International Centrifuge Models SR 3 and PR 2 and the type Major MSE centrifuge) containing 65 ml f sterile 4 % trisodium citrate or 75 ml of ACD-solution formula A (USP) The needles for drawing the blood are of stainless steel with a tip of the Fenval type 1.8×60 mm, inner-polished, inner diam. 1.55 mm. They are attached to a 40 cm poly vinyl chloride tubing, 3 mm inner and 4 4 mm outer diam The PVC tubing is fixed to a plastic cannula with a steel tip penetrating the stopper of the bottle This aggregate is only used once The centrifuge bottles, containing 2 ml of 0 15 M aqueous sodium chloride, are sterilized in a steam autoclave and then filled with 65 ml of 4 per cent sterile trisodium citrate solution ($Na_3C_6H_5O_7 \times 2\ H_2O$) previously adjusted to pH 6.8 with concentrated hydrochloric acid. This procedure has been adopted because autoclaving of the bottles with sodium citrate will remove the silicone film. The blood i centrifuged within one to 2 1/2 hours at 1800— 2000 RPM for 60 minutes at +4 C. The plasma is siphoned off (Fig. 1) into siliconized centrifuge bottles, which have been sterilized (containing 2 ml of 0 15 M aqueous sodium chloride solution) in a steam autoclave and is again centrifuged at 2100—2300 RPM for 20 minutes at +4 C.

The blood cells must be removed from the plasma before fractionation. When the plasma is recentrifuged after it has been siphoned off from the bulk of blood cells, a definite increase is found in the mean *in vivo* activity of the final fraction I—O as compared to the series in which the blood has been centrifuged only once. After the second centrifugation the plasma is aseptically siphoned into a measuring burette, and then allwed to run into the fractionation vessel (Fig. 2)

) Siliconized 400 ml infusion bottles. Sca d navian Standard type 613, manuf ctured by Limmareds Glasbruk, Sweden.

way when hysterectomy was performed on a girl suffering from v Willebrand s disease (15)

Following these first communications about the results obtained with the concentrated AHF preparation Dr Nilsson and/or Dr M Blombäck were consulted by colleagues all over the country whenever haemophiliacs or patients with v Willebrand s disease were to be treated or haemorrhagic complications were to be analyzed Consequently the demand for the product far exceeded the capacity of the fractionation unit which could supply about 15 batches monthly from a total 120 donors A number of batches had to be given on vital indications often even without sterility tests. No accidents occurred however and the demand continued to increase This being the case, the project had to be enlarged.

In connexion with the 150th Anniversary of Karolinska Institutet in 1960 Knut och Alice Wallenbergs Stiftelse Stockholm donated to the Institute a sum of 1 8 million Sw ers., to be used for the erection of a new research building A committee consisting of Professor Sten Friberg, Professor Hugo Theorell and Professor Sune Bergström supervised the designing and construction of the building

In December 1960 the building was taken over by four research groups, one of them being the plasma fractionation unit. Totally 360 m² of floor area in two storeys (fractionation storey is shown in Fig 15) were reserved for it For the purchase of equipment for the blood-coagulation laboratory the same foundation donated 140 000 Sw ers in 1961 to B and M Blombäck Syskonen Wesséns Stiftelse Stockholm made a sum of 210 000 Sw.crs available for the same purpose An other large International Equipment refrigerated centrifuge SR 3 additional freeze-drying equipment ($ 6 000) and a steam autoclave ($ 4 500) (22 000 Sw.crs) were purchased A separate room was reserved within the unit for compressors vacuum pumps and electric machinery A cold room 2 × 4 m (—5 C) allowed work under aseptic conditions In addition there was a larger cold room 4×6 m (—25 C) On this occasion as on previous ones, the installation of the equipment was supervised by Mr Nils Grondahl

The capacity of the new unit allowed blood from 300—350 donors to be worked up monthly in daily batches, each from 10 to 30 donors Larger batches have not been prepared because of the risk of hepatitis which however is not particularly great in Sweden In spite of more than 950 administrations of whole or half doses, i.e. fraction I—O prepared from 1.500 and 750 ml of plasma respectively hepatitis occurred only in two patients and one employee One of these patients received 165 blood transfusions during the same period of treatment

A fairly well equipped laboratory could now take over the routine manufacture of the concentrated AHF preparation simultaneously offering space for research work and analyses of coagulation factors in the blood

Furthermore in order to assure undisturbed continuity of the research work in the blood coagulation field an associate professorship in coagulation research attached to the Chemistry Department II was established at the Institute in 1960 to which post

Birger Blombäck was appointed on May 1 1961

Preparation of Fraction I—0

Since a good yield of AHF cannot be obtained after sterile filtration (through sintered glass) of fraction I—O the whole fractionation procedure has been performed in a closed system under aseptic conditions.

Blood collection In order to minimize the risk of contamination with hepatitis virus, initially plasma from only 8 donors was pooled each time (175—225 ml of non-lipaemic plasma from each donor) all the donors belonging either to the same ABO blood group as the patient or to the O group Later 10, 16 or 30 donations were used for each batch, and no attention was paid to the blood group of the patients, but the donors for each particular batch belonged to the same ABO group

A small sample of blood is drawn into a test tube at the end of blood letting If from the colour of the plasma suspicion of liver disease is aroused, the plasma in question is not included in the pool. The donors are instructed to report immediately to the Blood Transfusion Centre on appearance of any signs and symptoms compatible with early hepatitis.

400—440 ml of blood are drawn in the course of about 6 minutes, with careful manual stirring, into siliconized centrifuge bottles) (fitting the International Centrifuge Models SR 3 and PR 2 and the type Major MSE centrifuge) containing 65 ml of sterile 4 % trisodium citrate r 75 ml. of ACD-solution formula A (USP) The needles for drawing the blood are of stainless steel with a tip of the Fenval type 1.8×50 mm, inner-polished, inner diam. 1.55 mm They are attached to a 40 cm polyvinyl chloride tubing 3 mm inner and 4.4 mm outer diam. The PVC tubing is fixed to a plastic cannula, with a steel tip penetrating the stopper of the bottle This aggregate is only used once. The centrifuge bottles, containing 2 ml of 0.15 M aqueous sodium chloride, are sterilized in a steam autoclave, and then filled with 65 ml of 4 per cent sterile trisodium citrate solution ($Na_3C_6H_5O_7 \times 2\ H_2O$) previously adjusted to pH 6.8 with concentrated hydrochloric acid This procedure has been adopted because autoclaving of the bottles with sodium citrate will remove the silicone film. The blood is centrifuged within one to 2 1/2 hours at 1800— 2000 RPM for 50 minutes at +4°C. The plasma is siphoned off (Fig 1) into siliconized centrifuge bottles, which have been sterilized (containing 2 ml of 0.15 M aqueous sodium chloride solution) in a steam autoclave, and is again centrifuged at 2100—2300 RPM for 20 minutes at +4°C.

The blood cells must be removed from the plasma before fractionation. When the plasma is recentrifuged after it has been siphoned off from the bulk of blood cells, a definite increase is found in the mean *in vivo* activity of the final fraction I—O as compared to the series in which the blood has been centrifuged only once. After the second centrifugation the plasma is aseptically siphoned into a measuring burette, and then allwed to run into the fractionation vessel (Fig 2)

) Siliconized 500 ml infusion bottles. Scand avian Standard type 612, manufactured by Limmareds Glasbruk, Sweden.

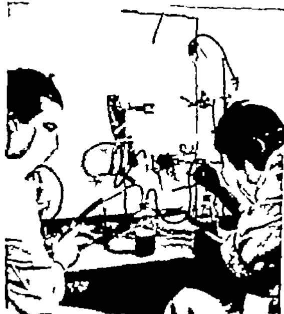


Fig. 1 After the first centrifugation of the blood the plasma is sucked off into siliconized centrifuge glasses for the second centrifugation

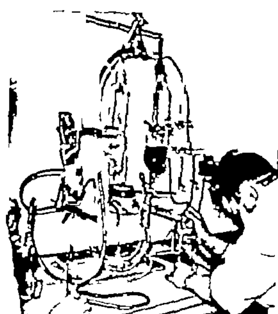


Fig. 2. After the 2nd centrifugation of plasma the clear plasma is drawn up into the measuring cylinder on the right The plasma then passes into the fractionation flask below Ethanol and buffer are added to the measuring cylinder on the left

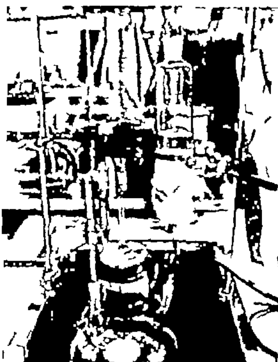


Fig. 3 Precipitation of fraction I in the cold bath Ethanol with buffer is added from the measuring vessel on the upper right.

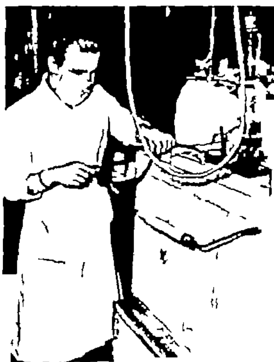


Fig. 4 Cotton filtered air under pressure is led into fractionation vessel to press fraction I + supernatant I into centrifuge cups.

Fraction I is precipitated from plasma essentially according to Cohn's method 6 (Cohn *et al* J Am Chem. Soc. 68 459 1946)

Cohn's description. The plasma is stirred gently but thoroughly and cooled as quickly as possible to 0 without permitting the formation of ice

The stirring is continued while sufficient sodium acetate-acetic acid buffer in 53.3 lum per cent (at 25 C) ethanol-water mixture is added through c pillary jets t bring the pH to 7.±0.2 and the final ethanol concentration of the system to 8 per cent. The additi n rate is 50—100 cc. per jet per minut and the over-all time for the addition should be about ne and one-half hours. During the addition the temperature is allowed to fall so that the system is mai tained close to its freezing point and so that the final tempera ture is between —2.5 and —3 This first tep requires 0.177 liter (measured at —5) f 53.3 per cent ethanol for each liter of plasma (measured t 0°) and about 1 cc. of 0.8 molar sodium acetat buffered t pH 4.0 with acetic acid for each liter of plasma should suffice f the pH djustment. The buffer has m lo rati of sodium acetate to acetic acid of 0.2 and is conveniently made up by taking 200 cc. of 4 M sodium acetate, 400 cc 10 M ceti acid and water to mak 1 liter (This buffer diluted with w ter eighty times should have a pH of 4.00 ± 0.02 when measured with a glass electrode at 25)

Cohn technique has been modified in the following way Sterile ethanol precooled to —10°C, containing buf fe is added dropwise during 15—30 minutes with constant stirring (Fig 3) D ring the addition the temperature must not exceed —1—0 C. If sod um citrate is used as anticoagulent, 1.2 ml of acetate buffer is added to 1— ml of sterile 53.3 % ethanol, i.e per liter of citrated plasma. If ACD-plasma is used the amount of buffer will be between 0 —1.0 ml. After addition of ethanol, the tem perature is decreased to —3 —4 C while the suspension is stirred for 30 minutes and left standing at — 3 C for another 4 minutes without stirring. The contents of the flask are then pressed over aseptically (Figs. 4, 5, 6) into welded centrifuge cups of acid-proof stainless steel (190—200 mm high, 100 mm outer diameter 1.25 mm thickness, weight 750 g) They are supplied with a lid of the same material (inner diameter 101 mm, height 20 mm, weight 300 g) designed in such a way that the contents of the centrifuge cup can be sealed off from the surrounding air by screwing on the *lid* As an extra precaution the closed centrifuge cups are kept in a plastic bag during centrifugation The fraction I suspension is centrifuged (for 20—25 minutes at —4 C) and 2400 RPM After centrifugati n the lids are removed from the centrifuge cups in a closed aseptic chamber where the centrifuge cups are main tained at a temperature of —3 C, and supernatant I is sucked off into a closed sterile container (Figs 7 8)

Extraction 1 If the fraction I suspension has been centrifuged in more than one cup, the precipitates from up to 4 cups are transferred into one single cup by means of a glass spatula. (Fig. 9) Extraction solution 75 g of glycine (p a.) and 1.62 g. of trisodium citrate are dissol ed in sterile and pyrogen-free distilled water to a volume of 935 ml. Concentrated hydrochloric acid (about 0,3 ml) is added to adjust the pH to 6.8 On the day of fractionation 65 ml of sterile filtered



Fig 5 Fraction I + supernatant I are pressed over from the *fractionation* vessel into centrifuge cups (on right)

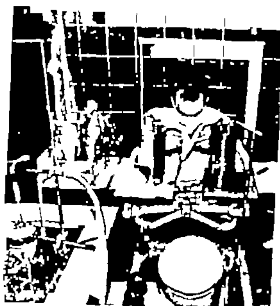


Fig. 6 During the last part of filling the centrifuge cups are balanced

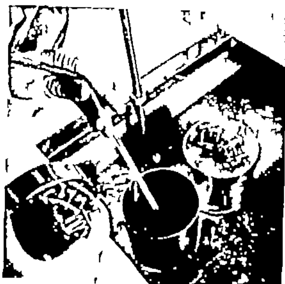


Fig 7 After centrifugation of fraction I the lids are removed from the centrifuge cups in a closed aseptic chamber and supernatant I is sucked off into a closed sterile container (Fig 8)



Fig 8. Collection of supernatant I.

absolute ethanol (p a) is added to this reagent For the extraction with the aqueous glycine-citrate-ethanol mixture a total volume of 1/4 the starting plasma volume is used The extraction solution is chilled to —3 C before use A very small portion (1/20—1/30) of the extraction buffer is added and the precipitate triturated and homogenized to a smooth paste, using a glass spatula The rest of the extraction buffer is



Fig. 9 The fraction I paste is transferred t one of the metal cups for extraction (still in th aseptic chamber)



Fig. 10 A lid with built-in stirrer is locked on the metal cup before extraction of fracti n I



Fig. 11 Extraction of fraction I i progress.

added. A lid with an inserted stainles teel tirrer (Figs. 10 1., 13) i now screwed on to the centrifuge cup and the su pension mechanically stirred during 30 minutes (Fig. 11)

Thereafter the lid with the stirrer is replaced by a plain lid and the suspension centrifuged for 12 minutes at 2500 RPM and —4 C. After centrifugation the upernatant is sucked off in the same way as the supernatant of fraction I

The *second extraction* is performed under the same conditions as the first extraction except that the time for centrifugation s somewhat longer i.e. 15 minutes. The residue after the second extraction, *fraction I—0* is *dissolved* in chilled (+4 C) terile 0.055 M sodium citrate solution, pH 6.8 The total volume should be about 1 8 that of the original plasma volume. A small portion of the buffer is first added and the precipitate fragmented whereupon the rest of the buffer is added. The cup is provided with a lid with inserted stirrer and transferred to a water bath f +30°C. The precipitate i usually in solution after stirring for

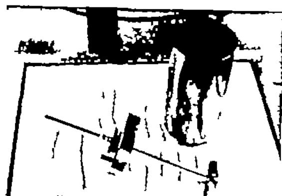


Fig 1 Centrifuge cup + assembled lid with stirrer in place

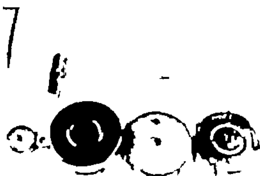


Fig. 13 Disassembled lid of centrifuge cup with stirrer Inner section of lid (far right) fits under two flanges on the inside of the centrifuge cup When the packing (centre) and outer lid section (*left*) are tightened in place by screwing on the small ring to the extreme left an air-tight seal is formed The stirrer fits into the central hole tightened by teflon packings. The excentrically located hole is connected to a sterile cotton wool filter for equilibration with atmospheric pressure

Prior to centrifugation the lid is replaced by another lid, identical in design except that the stirrer has been removed and both the central and excentric holes have been closed by attaching a short piece of plastic tubing sealed by bending back on itself and secured by wire

30 minutes The solution is transferred aseptically to a measuring cylinder passed through a fine nylon cloth and distributed in 100 ml. portions into sterile non-siliconized glass bottles. (300 ml infusion bottles Scandinavian Standard Type 013 manufactured by Limmared s Glasbruk [= glassworks] Sweden) A smaller volume 10—20 ml for various tests is taken into a separate bottle The rubber stoppers are replaced by specially designed sterile cotton filters. The bottled solutions are then *rapidly shell frozen at about —70 C* and freeze-dried under aseptic conditions, the pressure in the cabinet during freeze-drying being 0 1—0 05 mm Hg (Mc Leod) The pressure after freeze-drying is usually 0 01—0 03 mm Hg

After freeze-drying is completed nitrogen or air is let into the freeze-drying apparatus through silica gel and a cotton filter The individual cotton filters of each bottle are exchanged for rubber stoppers and the freeze-dried preparations are stored *at —20 C until used The contents of* the smaller bottle are always tested for sterility by culture for contamination with tetanus by injection into guinea pigs for toxicity in mice and *occasionally also for pyrogenicity* in rabbits.

Solution and administration of fraction I—0 Each bottle of fraction I—0 contains from 1 4 to 1 7 g of protein and 1.5 g of sodium citrate in addition to a small quantity of glycine and sodium chloride When the dry powder is dissolved in 100 ml of distilled water the solution will be isotonic with the blood and the pH 6 8

When the bottle containing the freeze-dried AHF preparation has assumed room temperature 100 ml of sterile pyrogen free distilled water is added preferably by means of a sterile transfer set

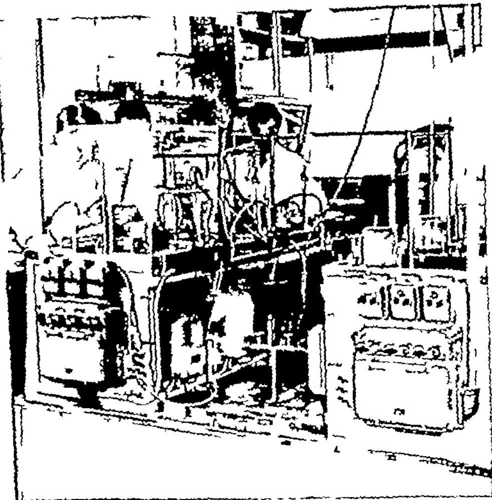


Fig. 14. The fractionation unit.

The bottle is rocked gently until all substance has gone into solution, which takes about 20—30 minutes. To avoid foaming the bottle should not be shaken. Once in solution, the sample must be administered within 1—2 hours.

The AHF preparation may be sensitive to certain metal ions. Therefore should it be transferred for some reason to another bottle a metal syringe should not be used. The cannulas must be of stainless steel.

The preparation is given intravenously with an *apparatus* of the customary type for blood *transfusion*. To avoid waste the infusion set is first filled with sterile physiological saline and then adapted to the inverted AHF bottle. The solution is given slowly one bottle (100 ml) in the course of 15—30 minutes.

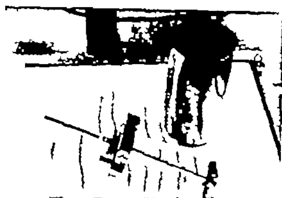


Fig 12 Centrifuge cup + assembled lid with stirrer in place.

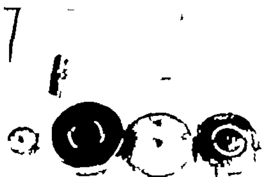


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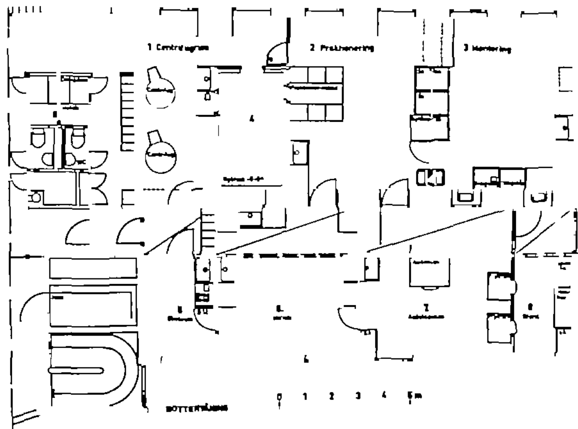


Fig. 15 Floor plan the new pilot plant laboratory for cold fractionation

1 Centrifuge room
2. Fractionation laboratory
3 Supply room
4 Cold room
5 Digestion room.
6 Washing room
7 Sterilizing room
8 Sterile room

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18 Nilsson I M and Blombäck, M. Treatment of haemophilia A with human antihemophilic globulin. Acta Med Scand. *161* 301—321 1958.

Fractionation record

Antihaemophilic Factor

Constitution of Pool

Run No
Date May 10

No.	Group	Name	Comments

Antihaemophilic Factor

Fractionation performed by A N
N N

Prep No
Date *May 10*

Donations used *16*
435 mls. blood drawn into *65* ml of *4* % *Na_3Ci* in siliconized bottles
Stirred by *hand (all magnet shaker)*
Blood drawn between *8 a m.* and *9 30 a. m.*
Average bleeding time per donor *6* minutes
No of centrifugations *two*
 Blood at *1 800* R P M (SR 3 or MSE) *50* minutes + *4* C temp
 Plasma at *2 000* R. P M (SR 3 or MSE) *0* minutes + *4* C temp
Plasma available at *1 30 p m*
Appearance of plasma clear (all yellowish, haemolysis lipaemia)
Comments *Yellowish and heavily lipaemic plasmas are discarded*

Fraction I

Plasma Volume *3 748* ml
53 3 % Ethanol required (0 177 l. per l of Plasma) *664* ml
 (Prepared *March -61*).
Buffer A required (1 ml per l of Plasma) 4 5 ml
 (Prepared *Feb -61*)
Addition Started *2 15 p m.* temp 0 — 1 C.
 Completed *2 35 p m.* temp *0* — 3 C.
  Time for addition *20* minutes
  Stirred for 30 minutes at 3° — 4 C.
  Allowed to stand for 45 minutes at 3° — 4 C.
pH *of supernatant 1* (7 2 ± 1)
Centrifuging Started *4 p m.* temp 4 C. 2 500 R. P M (SR 3)
 Completed *4 20 p m.* temp 4 C.
 Volume of supernatant — — — ml.
 Samples × *2* ml
Comments *Buffer A = fraction I — buffer of method 6 of Cohn et al J ACS 68 459 1946)*

Extractions

Extraction E 1
Volume of glycine *Buffer required (1/4 plasma volume) 0 940 l*
 (Buffer prepared *April 61*) Temp 4 C.
 Suspension started *4 30 p m.*
 Completed *4 40 p m.*

Stirred for 30 minutes. Temp 3° — 4° C.
Centrifuging started 4.20 p m. Temp 4° C. 2 500 R. P M. (SR 3)
completed 4.35 p m. Temp. 4° C. 15 minutes
Volume of supernatant 860 ml.
Samples 2 × 2 ml.

Extraction E- 2
V lume of glycine Buffer required (1/4 plasma volume) 0 940 L
(Buffer prepared April -61). Temp 4 C.
Suspension started 5 40 p m.
completed 5 40 p m.
Stirred for 30 minutes. Temp 3° — 4° C.
Centrifuging started 6.25 p. m. Temp 4° C. 2.800 R P M. (SR 3)
completed 6 45 p. m. T mp. 4° C. 15 minutes.
Volume of supernatant 850 ml.
Samples 2 × 2 ml.

Solution of I—O

V lume 1 0 0.05 M Sodium Citrate Buffer required (1/8 Plasma Volume) 495 ml. + 4 C.
(Buffer prepared April -61).
Stirred for 30 minutes at 30° C.
Filtered through nylon filter in dropping chamber
Transferred t 6 bottles.
Bottle 1 100 ml
2 100 ml
3 100 ml
4 80 ml
5 80 ml
6 ml
Samples 10 ml Sterility and t xicity
10 ml Pyrogen
6 × 2 ml Activity
Containers Sh ll Frozen at 70° — 80° C.
Activ ty Samples Stored at 20° C.
Freeze Drying Started May 10 Date 10 p m. Time Vac. 40 Microns Hg.
Completed May 13 Date 10 a. m. Time Vac. 8 Microns Hg.
Comments Total time for fractionation 14 hours.

Results of Testing

A. H F Acti ity per ml. (Volume before drying) 800 / ml
Test system used / (Acta Med Scand. 159 33 1957 184 357 1950)
F brinogen Protein 16 9 mgm./ml
Coagulabil ty 89
Fibrinogen 14 mgm /ml
Pyrogen Not performed
T xicity No
Sterilit Yes
Other tests
Bottling Record No
Details of Issue To. V V Haemophil A pati nt The General Hospital, Malmö
Date
Time of despatch
Comments

Acta Medica Scandinavica, Supplementum 379 1962

From the Chemistry Department II (Head Erik Jorpes, M.D.), Karolinska Institutet, and the Department of Medicine I (Head Erik Sköld, M.D), S:t Eriks Sjukhus, Stockholm, Sweden

The Haemophilia Situation in Sweden

by

Erik Jorpes and Olof Ramgren

In Sweden, nearly all the haemophiliacs are registered In 1944 Erik Sköld (13) of S:t Eriks Sjukhus, Stockholm made the first genealogical and medical survey of these patients.

Starting in 1942 he organized blood transfusion centres in different parts of the country and put the haemophiliacs in touch with hospitals having facilities for blood transfusions. Special schools and vocational training were organized for haemophiliacs at the institutions for handicapped persons, and attempts were made to inform the carriers, both definite and genetically potential, about the risk of propagating the disease by giving birth to new haemophiliacs. The importance of birth-control and sterilization of both haemophiliacs and carriers was stressed

During the period 1900—1942, the average length of life of persons with severe haemophilia in Sweden was 16.5 years. The corresponding figures for moderate and mild haemophiliacs (A+B) were 19.9 and 29.6 years (10) The medical care given, primarily blood transfusions and general supervision, then prolonged the average length of life of the haemophiliacs during the period 1943—1957 to 23.2 40.1 and 50.6 years, respectively for the severe moderate and mild forms, *i. e.* to approximately 1/3, 1/2 and 2/3 of the normal life expectancy for males during that period (10) There was also a change in the clinical picture intracranial and intestinal haemorrhage being the most common causes of death during the latter period, as compared to post-traumatic and internal haemorrhage during the former

In recent years, particular attention has been paid to these haemorrhagic diatheses. A new medical survey has been made the carrier problem (6, 7) has been dealt with and

pation and lived solely on a disablement pension and social benefits.

Both Ikkala (2) and Ramgren (10) emphasized suitable vocational guidance and training from an early age, as being of utmost importance in improving these persons ability to earn a livelihood In the series collected i Sweden there are several examples of severe haemophiliacs earning a full livelihood in intellectual occupations or business. When the intellectual capacity does not permit academic studies, another type of suitable training must be given at an early age

An analysis was made of the length of hospitalization required and the number of blood transfusi ns received by these patients. During the period 1943—1957 a severe moderate or mild haemophiliac had to spend an average of 1 0 0 7 or 0.3 weeks in hospital annually and received 3.9 1 6 or 1.2 blood transfu ion units, respecti ely Q ite a number of them had to spend about one month a year in hospital

Particular ttention ha been paid to the *hereditary pattern of the disease* (Nil son Blombäck, Ramgren & \ Francken (6) 1962 and Ramgren Nilsson & Blombäck (9) 1962) *Since no difference was f und be tween haem philia A and B from the clinical point of view (Ramgren (8) 1962) the two diseases were treated togeth r* The family history of 180 families wa tudied (9 11)

A total 173 families could be traced thro gh three or more generations a positi heredity wa demon trated in 127 of them, *i e* in 73 per cent. Coagul tion studies in 14 families howed that 113 of them had haemophilia A and 32 had haemophilia B (22 per cent) In 39 families, two or more haemophiliacs were investigated. *All the affected members of an individual family were found to have the same clinical form of haemophi lia — severe moderate or mild*

In 69 families *i e* 38 per cent, there had been only one affected member A total of 70 families, i e 44 per cent, had two to four haemophi liacs. The remaining 32 families, *i e* 18 per cent each had five or more affected members. In two families there had been 13 and 14 haemophiliacs, respectively Almost all the cases had been diagnosed by Sköld, Nil son or Blombäck

Twenty-six of the 180 families had no living haemophilic member at the time However as far as could be ascertained, only four families had no potential carrier of fertile age.

During the past three decades (1931—1960) 19 living definite or probable carriers, 81 pc tential carriers with a 50 per cent risk and 61 potential carriers with a 25 per cent risk were born in the families with clinically severe haemophilia. Probably there re at p esent 75 carriers of fertile age in these families. The cor responding number of carriers of cli nically moderate and mild haemophi lia is 29 and 54 respectively

In surveying the haemophilia si tuation in Sweden A and B taken together Ramgren (10) finds that no reduction in the incidence of the disease has occurred (Fig 1)

During the period 1931—1955, one haemophiliac was born per 8,000 li e born male infants, giving one living haemophiliac per 13,000 men, about the same frequency as found in Denmark (1 14,000 Sjølin (12) 1960) and in Finland (1 13 000—14 000 Ik kala (2) 1960) Since only half of

the genealogical (9) and medico-social aspects have been re-investigated (10) New therapeutic possibilities have been opened up through the use of the purified fibrinogen fraction I—0 of Blombäck & Blombäck which contains factor VIII Bleeding episodes in haemophilia A patients (1 5) and in cases of v Willebrand s disease (4) have been treated and various surgical procedures have been performed under protection of this preparation Detailed reports of the results have been given by I M Nilsson B Blombäck M Blombäck and O Ramgren.

In Sweden s population of 7 million there are about 300 or more likely 350 living haemophiliacs belonging to about 155 families The higher figure includes a number of mild cases with mild symptoms Of this number *176 belonging to 130 families* are well analyzed as to the type and form of haemophilia. Twenty two per cent of them have haemophilia B Of the 101 haemophilia A families with 133 cases 57 per cent are of the severe form (AHF activity as determined on *haemophilia A plasma* in a recalcification system less than *1 per cent) 18 per cent are moderate* (AHF activity 1—5 per cent) and 25 per cent are mild (AHF activity 5—25 per cent) The corresponding figures for the distribution in the 29 haemophilia B families (43 cases) are 41 28 and 31 per cent respectively

Since the fate of a haemophiliac is not only of personal concern but also a matter involving the community Ramgren (10) made an analysis of the present educational and occupational situation of 235 haemophiliacs *112 of* whom were of the clinically severe form 45 moderate and 78 mild Thirty-six of them had not yet reached school age.

Forty per cent of the 68 severe haemophiliacs attending ordinary schools could not keep up with the course of *study because bleeding* episodes necessitated frequent long absences. Twenty five of the *severe haemophiliacs* received special education at home or at boarding school The educational standard reached and the choice of occupation proved to have a decisive influence on the social status of the haemophiliacs. The total *13 haemophiliacs of all degrees of severity* engaged in *intellectual work e g* engineers, journalists and office managers, had a good earning potential Only two one severe and one mild haemophiliac of 24 engaged in *trade or doing light work* such as *business men clerks watch makers* and opticians, could not earn their living by their occupation In the group doing *light manual work* such as light factory workers, waiters, electricians, book-binders and printers assistants, only 5 of 21 severe haemophiliacs *i e* 24 per cent were self supporting The corresponding figures for the moderate and mild forms were 47 and 94 per cent The two severe haemophiliacs doing *heavy manual work* both farmers, could not earn their living Of the two *moderate haemophiliacs doing* heavy manual work, one (a construction worker) could earn his living but the other (a lorry driver s assistant) could not Nor could 13 of 17 *i e., 76* per cent in the group of *mild haemophiliacs*

Out of 129 haemophiliacs, a total 16 (12 severe 2 moderate and 2 mild) *i e.,* 12 per cent had no occu

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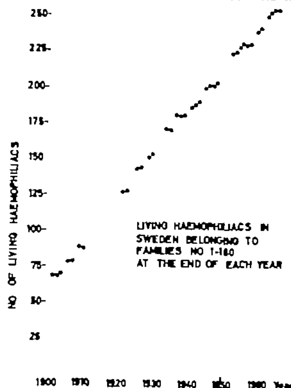


Fig 1 Living haemophiliacs in Sweden belonging to families 1—180 at the end of each year

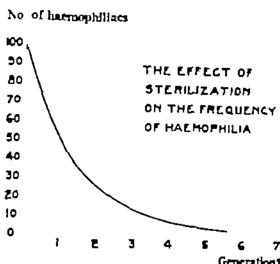


Fig. 2. The effect which the sterilization of the male *haemophiliacs would have* on the frequency of haemophilia (E. Sköld 1944)

the mild cases are diagnosed Ramgren estimates a total of 342 haemophiliacs, an incidence of one living haemophiliac per 11 000 men

Some Medico-Social and Eugenic Aspects of the Therapy of Haemophilia

Many of our modern therapeutic achievements must from time to time prompt the physician himself to question their wisdom By preserving the life of a patient up to fertile age he may preserve and perpetuate metabolic errors and constitutional deficiencies a result not uncommon in haemophilic families Being dedicated to the preservation of life his situation was a most unhappy one in *the pre-transfusion era when even* heirs to royal crowns could bleed to death He would certainly have accepted a specific remedy for haemophilia as enthusiastically as we greeted insulin and the antibiotic drugs

In view *of the fact that hereditary deficiencies cannot yet be aetiologi*cally corrected, one cannot be too optimistic about the immediate future of haemophilia therapy We will certainly be able to make most of the known blood-clotting factors clinically available for occasional use in bleeding emergencies and for surgery in haemophiliacs. But this will not suffice A haemophiliac will still be a haemophiliac, unable to enjoy life freely during childhood and unfit to cope successfully with the rigours of adult life No substitution therapy *will make him normal*

Haemophilia will always be a prob-

lem for the affected individual, for his family and for the community In its severe form it means a catastrophe for the family a fact of which the haemophilic families themselves are well aware The care of the haemophiliacs has always been something of a nightmare for the sur geons. The patient often enters hospital with symptoms simulating a condition demanding major surgery Usually it i actually an internal bleeding, which hould be treated with a plasma con centrate. Even tooth extractions and minor surgical operations can be extremely hazardous, if performed without first raising the AHF level adequately

To a certain extent these difficul ties can be coped with if an active plasma fraction can be made available A previously reported (3) a pilot plant for the manufacture of a human plasma fraction which contains the antihaemophilic factor A has recently been establi hed at the Chemi try Department II of Karolinka I titutet the Stat Medical School in Stockh lm By December 1961 about 800 lots of human fraction I—0 each from 8 d nors, had been used in the treatment of haemophilia A and v Willebrand's disease

A t tal f 63 haemophiliacs had been treated on 227 occasi ns in con nexion with bleeding episodes or operation The treatment of haemophilia A has thereby been greatly facilitated But new impl ications will foll w

According to existing legislation in Sweden, patients with diseases requiring replacement thera y e g diabetes, Addison s disease pernicious anaemia, hypoparathyroidism and agammaglobulinaemia are provided with the required pharmaceutical products free of charge. The same applies to patients with haemophi lia. In the hospital as well as in the out patient departments, they receive both blood and plasma transfusions practically free of charge In the long run, this will cause a demand for the plasma fraction as well which it will be difficult to meet

From the scientist s point of view it is of course gratifying to be able to purify from normal human blood the very component which these patients lack. But there are other ways of mastering the situation, the most rational one being to limit the number of haemophiliacs by controlled parent hood It could even be considered that the physician only wholly fulfills hi mission by protecting future genera tions from being burdened with in herited health deficiencies. The question can also be raised as to what ex tent a citizen has the right to propa gate his disease thereby increasing the future burdens of society

The situation of the haemophiliacs can of course be looked upon from different points of view A stated by Quick in hi monograph Haemorrh agic Diseases (Lea & F biger Philadelphia 1957 p 159) "The questi n whether a carrier should be deferred from marrying and having children is serious and difficult to answer" In taking the most optimi tic view he tates that Had Queen Vict ria known that she was a carrier and had *foregone marriage* England would n t have it beloved royal family and their graciou Queen Elizabeth II Furthermore over 85 normal and 10 hemophilic descendants would not *have been born*. Perhaps, if the Tza revich had received the medical care that is now possible Ra putin would

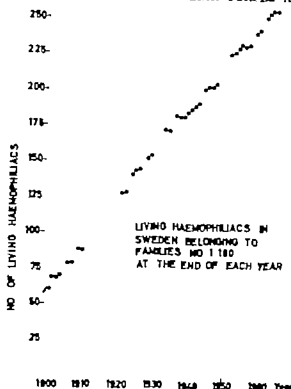


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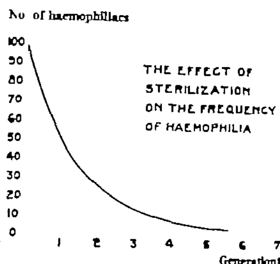


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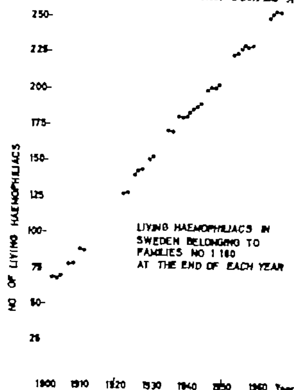


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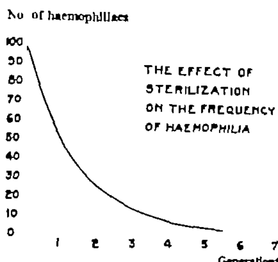


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a certain risk of bleeding in connexion with minor or major surgery.

Any action taken by the physician must, however be in accord with the prevailing religious and ethical concepts of the society. He will have to consult and, if possible, co-operate with the authorities in these fields.

In fact, such a consultation on the highest level took place when, on Sept. 12th, 1958, the members of the 7th International Congress of Haematology in Rome visited the Head of the Catholic Church, Pope Pius XII. The Holy Father took a sympathetic attitude to the many questions presented to him by the visitors, especially those concerning the control of parenthood in cases of inherited mental and physical health deficiencies. Grateful for being consulted concerning the ethical aspects of the problems, he replied that most of the questions under discussion were primarily of medical nature.) Après avoir discuté les solutions proposées, couramment, au problème de l'hérédité défectueuse, il Nous reste encore à donner réponse aux questions que vous Nous avez posées. Elles s'inspirent toutes du désir de préciser l'obligation morale découlant de résultats de l'eugénique que l'on peut considérer comme acquis.

Il s'agit, dans les différents cas présentés, de l'obligation générale d'éviter tout danger ou dommage plus ou moins grave tant pour l'intéressé, que pour son conjoint et ses descendants. Cette obligation est proportionnée à la gravité du dommage possible, à sa probabilité plus ou moins grande, à l'intensité et à la proximité de l'influence pernicieuse exercée à la gravité des motifs que l'on a de poser des actions dangereuses et d'en permettre les conséquences néfastes. Or ces questions sont en majeure partie des questions de fait, auxquelles seuls l'intéressé le médecin et les spécialistes consultés peuvent donner réponse. Au point de vue moral on peut dire en général que l'on n'a pas le droit de ne pas tenir compte des risques réels que l'on connaît.²)

His willingness to give the medical authorities the final decision was still more clearly pointed out in the following sentences concerning the risk involved in Rh incompatibility: "Dans le cas d'un couple en situation Rh vous demandez aussi s'il est permis de déconseiller toujours la pro-

) Cited from L'Osservatore Romano, Città del Vaticano Sept. 15—16, 1958. The English translation is taken from "Two discourses by Pope Pius XII" Bulletin No. 11 1959 of The Dight Institute of the University of Minnesota Uni of Minn. Press, Minneapolis.

) Now that We have discussed the solutions that are currently proposed for the problem of defective heredity it remains for Us to reply to the questions which you submitted to Us. These questions are all inspired by the desire to define precisely the moral obligation which is consequent upon those conclusions of genetic science which may be regarded as settled.

In all the cases presented, the question concerns the general obligation to avoid all more or less serious danger or harm either to the individual or to his partner and descendants. This obligation is proportionate to the gravity of the possible harm, to its greater or lesser probability to the intensity and proximity of the pernicious element which is at work, to the gravity of the reasons one has for placing acts which inspire danger and permitting their disastrous consequences. For the most part these are questions of facts, to which only the individual concerned the doctor and the specialists who are consulted can give a reply. From the moral standpoint it may be said in general that a person has no right to disregard genuine risks of which he is aware.

have remained an obscure monk The other side of the Royal coin shows what could have been avoided There we see Prince Gonzales of Spain dying at the age of 20 from haemorrhage, and his brother ex Crown Prince Alfonso dying at the age of 31 after a not particularly se rious car accident in Miami Florida on Sept 6th 1938 In the picture we also see Kaiser Wilhelm II of Ger many doing his best to keep secret the fact that Princess Irene had in troduced haemophilia into his family his nephew Prince Heinrich dying at the age of four and the highly cul tured Prince Waldemar of Prussia dying from an internal haemorrhage after a rough truck ride when he tried to escape from Silesia at the end of World War II The haemophilia of the last Tzarevich completes the pic ture, a terrible blow to a family and of significance in the downfall of an empire comparable in size to the British

In their contributions to the Inter national Symposium on Hemophilia and Hemophilioid Diseases (Edit K. M Brinkhous, N Carol Univ Press, Chapel Hill 1957) A Pavlovsky Ar gentine and J Favre-Gilly France discussed the marriage problems and risk to the descendants of haemophi liacs, the medico-social aspects of the disease and the necessary steps to be taken regarding schooling and voca tional preparation and the medical care of the haemophiliacs. In these respects the situation is presumably about the same in all countries

As to the question of the ways in which hereditary transmission of the haemorrhagic diatheses should be avoided different views may arise Pavlovsky points out that even in a country where religious traditions have a fairly strong influence on po pular opinion the majority of the women carriers desired their children to marry but did not wish them to have descendants

As an obstacle to recommending sterilization of the carriers, Dr Pav lovsky mentioned the uncertainty of whether or not a woman was a car rier Such information was formerly often unattainable

By refining the technique for quantitative determination of factor VIII and factor IX in plasma I M. Nilsson and M Blomback recently showed that women carriers of fertile age always have a subnormal level of the specific coagulation factor acti vity (Nilsson Blombäck Thilén & v Francken (7) 1959 and Nilsson Blombäck Ramgren & v Francken (6) 1962)

A total 33 definite carriers of hae mophilia A 14 probable carriers (with one haemophilic son but no family history) and 32 potential carriers were examined Significantly low va lues for the antihaemophilic factor (AHF or factor VIII) were found in 34 of 35 definite or probable carriers of fertile age and in 16 of 32 poten tial carriers

A study was also made of 18 definite 5 probable and 18 potential carriers belonging to haemophilia B families. Low haemophilia B factor (factor IX) values were recorded in 20 of 23 de finite or probable carriers, and in 7 of 18 potential carriers. Observations along the same lines were recently made by Ikkala (2)

From this also follows that the wo men carriers of haemophilia A or B during the fertile age are to be consi dered as mild haemophiliacs, running

plagued with fear during all their fertile years that they may give birth to another haemophilic son. Their anxiety frequently spoils family life and is often transferred to the sisters of the haemophiliac, although they are only potential carriers with a 50 per cent genetic chance of being normal.

The situation will improve to some extent in the future when, as pointed out earlier, the physician presumably will be able to tell the presumptive carrier whether or not she is a carrier.

Fortunately, there is an increase in AHF activity during pregnancy. Therefore there is little bleeding tendency at abortion, if performed on carriers belonging to haemophilia A families. Furthermore, if the pregnancy goes to full term, there will be less haemorrhage at delivery attributable to low AHF activity.

As is evident from the recent survey of the haemophilic families in Sweden, types A and B taken together, the number of haemophiliacs in the community is increasing. Only 4 of the 180 haemophilic families studied had, as far as could be ascertained, no potential carrier of fertile age. Thus, in practically all the families there is a possibility of the disease being transmitted to the next generation.

In his thesis, Sköld pointed out that voluntary sterilization of the haemophiliacs, if strictly applied, could eliminate haemophilia in the course of 6—7 generations, assuming that no new cases appear by mutation, an occurrence which we consider extremely unlikely. In the Swedish series, direct inheritance was stated in 73 per cent of the known families, the "sporadic cases" being mostly elderly people whose family records were poor or non-existent.

However, as has been shown in the famous Swiss haemophilic family from Tenna, the gene of haemophilia has been known to skip five female generations. To trace such carriers in order to prevent propagation of the disease is almost impossible. They do not as a rule know their ancestors four or five generations back, and certainly not if these had bleeding symptoms. There will thus always be hereditary haemophilia showing itself as "sporadic cases". Their appearance will not be prevented by the above indicated means.

From the eugenic point of view there is no advice to give when the husband is a haemophiliac or the wife is a carrier, other than to refrain from having children. We have at present no means of telling an expectant mother who is a carrier whether or not her child will be normal. It has been claimed that prenatal sex determination could be of some help for these women. The decision would be to apply abortion in case of a male foetus. However, there is a 50 per cent chance that the boy will not have the disease. If the sex of the child *in utero* were female, there is a 50 per cent risk that the embryo is a carrier who will one day face the same situation as her mother. Consequently prenatal sex determination does not help in this situation. The eugenic solution is to abstain from parenthood.

Here medical considerations re-enter the discussion. The means of avoiding natural parenthood may be left to the judgement of the physician, who will perhaps also see fit to advise adoption of a normal child. In

création ou s'il faut attendre le premier incident?

Les spécialistes de la génétique et l'eugénique sont plus *compétents que* Nous en ce domaine. Il s'agit en effet d'une question de fait, qui dépend de facteurs nombreux, dont vous êtes les juges compétents. Au point de vue moral il *suffit d'appliquer les principes* que Nous avons exposés plus haut avec les distinctions nécessaires.³)

He acknowledged the value of dissemination *of medical information* "*Vous demandez enfin* s'il est permis de faire de la propagande sur le plan technique pour souligner les dangers inhérents au mariage entre consanguins. *Sans aucun doute,* il est utile d'informer le public des risques sérieux qu'entraînent les mariages de ce genre. On tiendra compte ici également de la gravité du danger pour juger de l'obligation morale.⁴)

Finally he paid his homage to the free scientific discussion in the following way: Avec sagacité et persévérance vous tentez d'explorer toutes les issues possibles à tant de situations difficiles; vous vous employez sans relâche à prévenir et guérir une *infinité* de souffrances et de misères humaines. Même si des précisions ou des modifications apparaissent souhaitables *en certains points, cela n'en* lève rien au mérite incontestable de vos travaux. Nous les encourageons bien volontiers. Nous apprécions hautement la collaboration active et sérieuse qui permet *aux diverses opinions* de s'exprimer librement mais *ne s'arrête* jamais aux critiques négatives. C'est la seule voie ouverte au progrès réel aussi bien dans l'acquisition de nouvelles connaissances théoriques, que dans leur application clinique."⁵)

With regard to haemophilia, the overshadowing problem is the question of how to avoid perpetuating the disease. The haemophiliac should not transmit it to his *grandsons,* and the woman carrier will have to take the same *responsibility* and abstain from having children of her own.

The women carriers, who have sons with severe haemophilia, are usually not only willing to abstain from *having further children but are often*

³) In the case of a couple in the "Rh situation" you ask *if it is permitted always to dissuade procreation or if it is necessary to await the first occurrence.* Genetic and eugenic specialists are more competent than We in this domain. Actually it is *a question of fact* which depends on many factors, of which you are the competent judges. From the moral point of view it is sufficient to apply the principles which We have explained above with the *necessary distinctions.*

⁴) You ask finally *if it is permitted to make propaganda on the technical level in order to emphasize the dangers inherent in a marriage between persons with blood relationship.* Without any doubt it is useful to inform the public of the serious risks which marriages of this kind entail. Here again account will be taken of the gravity of the danger in order to judge *the moral obligation.*

⁵) With shrewdness and perseverance you are endeavoring to explore all possible means of escape from so many difficult situations; you spend yourselves without respite in order to prevent and cure *numberless human sufferings* and miseries. Although some clarifications or modifications would seem to be desirable on some points, that does not detract from the incontestable merit of your labors. We most willingly encourage them. We deeply appreciate the active and serious cooperation which permits the free expression of different opinions but is not content with merely negative criticisms. *That is the only way* which opens on real progress, both in the *acquisition of new* theoretical knowledge and in its clinical application.

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his above mentioned address to the 7th International Congress of Haematology in 1958 Pius XII wholeheartedly gave his sanction to the adoption system which *offers the only safe outlook for a normal family life when one or both of the parents suffers from a severe inherited blood dyscrasia* Le dernier moyen mentionné plus haut et sur lequel Nous voulions exprimer Notre avis était celui de l adoption Lorsqu il faut déconseiller la procréation naturelle, à cause du danger d une hérédité tarée à des époux qui voudraient quand même avoir un enfant on leur suggère le système de l adoption On constate par ailleurs *que ce conseil est* en général suivi d heureux résultats et rend aux parents *le bonheur la paix* la sérénité Du point de vue religieux et moral l adoption ne soulève aucune objection c est une institution reconnue presque dans tous les Etats civilisés. Si certaines lois contiennent des dispositions inacceptables en morale cela ne touche pas l institution elle-même Du point de vue religieux, il faut demander que les enfants de catholiques soient pris en charge par *des parents adoptifs* catholiques la plupart du temps en effet les parents imposeront *à leur enfant adoptif* leur propre religion *)

In Sweden voluntary *sterilization* has during 1943—1960 been applied to three haemophiliacs *and 18 carriers*, in 13 of them in connexion with *abortion Most of the carriers* (12 cases) were potential carriers, sisters *of haemophiliacs*, who had from early childhood been confronted with the seriousness of the disease These women have as stated elsewhere a strong desire not to have haemophilic children Even if they have a 50 per cent chance of not being carriers, they definitely chose to adopt children instead of taking the risk of preserving *the disease in the family*

*) The last of the means mentioned above, on which We wished to express Our view was that of adoption. When it is necessary to advise against natural procreation because of the danger of tainted heredity in the case of married persons who would nevertheless like to have a child the system of adoption is *suggested to them. It has also been observed* that such advice usually brings good results and restores happiness, peace and serenity to the parents. From the religious and moral point of view *there is no objection* to adoption it is an institution which is recognized in nearly all civilized States. Although certain laws on the subject may *contain* provisions which are unacceptable from a moral point of view that does not affect the institution itself From the religious standpoint it is required that the children of *Catholics* be *cared for* by adoptive parents who are Catholics for it is a fact that in most cases parents will impose their own religion upon their adopted child

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PART II

Acta Medica Scandinavica, Supplementum 379 1962

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HAEMOPHILIA IN SWEDEN

V Medico-Social Aspects

By

Olof Ramgren

Haemophilia a haemorrhagic diathesis of hereditary nature and disabling course, has aroused great interest as far as its coagulation defects and hereditary pattern of sex-linkage are concerned. The medico-social aspects, which are of utmost importance to the individual haemophiliac, have not attracted research workers to corresponding degree. When studying haemophilia in Sweden (with I M Nilsson and M Blombäck) I have therefore investigated the medico-social aspects of the disease in the Swedish community

From the medico-social point of view the care of haemophiliacs in Sweden during the 20th century can be divided into three periods

Period I 1900—1942
Period II 1943—1957
Period III 1958 onwards

Before 1943, no special arrangements were made for the treatment of haemophiliacs, and blood transfusions were given only sporadically

Period II was initiated in 1943 by the work of E. Sköld who organized blood transfusion centres throughout the country and put the haemophiliacs in contact with hospitals which had facilities for administration of fresh blood. Special vocational training for haemophiliacs was organized t the institutions for the care of the handicapped, and attempts were made to inform the known carriers, as well as those identified as genetically possible carriers, about the risk of giving birth to new haemophiliacs. Education in birth-control was given, and

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sterilization of both haemophiliacs and carriers was performed

Since 1958, fresh plasma and new plasma derivatives including concentrated antihaemophilic factor (AHF or factor VIII) preparations have been available, but the results of this therapy from the medico-social point of view can not yet be assessed in view of the short period of time that has elapsed

Clinical Material and Methods

The present study was started in 1955 as a follow-up of Sköld's investigation of haemophilia in Sweden (19) Our aim was to study the results of transfusion therapy special vocational training and birth-control measures introduced by him in 1944 However by the time of our study the annual reports from the hospitals to the Royal Swedish Medical Board showed that several new haemophiliacs had been diagnosed during the years 1942—1957 At the end of 1960 the original 60 families studied by Sköld in 1944 had increased to 180 families

To obtain data for analysis, a questionnaire was sent to each known haemophiliac. The available case records were surveyed and a clinical examination was made of most of the patients either by me or by one of the collaborators (I M Nilsson and M Blombäck) A short case history was compiled for each haemophiliac, and is published together with the pedigrees (17) As shown in a previous paper on the symptomatology (15) there is no difference between the clinical features of haemophilia A and B of the same degree of severity Consequently haemophilia A and B are considered collectively in this paper

The questionnaire was as follows

I *Manifestations and nature of the disease*

What was the first sign of haemophilia?

At what age did the first bleeding symptoms appear?

Has profuse bleeding occurred
at dentation?
after tooth extraction?
after surgery?

Has haemarthrosis occurred
in ankle joints how many times?
„ knee
„ hip „ „ „
„ shoulder „ „ „
„ elbow „ „ „
„ wrist „ „ „
„ muscles „ „ „

Do bleedings into joints and muscles as a rule occur
spontaneously?
after slight trauma?
after severe trauma?

Have you ever had
haemoptysis? how many times?
haematemesis? „ „ „
melaena? „ „ „
haematuria?

Have the bleedings been most severe
at a certain season of the year?
at a certain age? In childhood or adulthood?

Have you had especially severe bleedings during certain years of your life?

Are you disabled by your haemophilia?

Which joints are most affected?

Do you have difficulty in walking?

Can you enter a tram bus or train without help?

Can you climb stairs without difficulty?

II *School attendance*

Have you been able to follow the courses in all subjects at school?

Have you been exempted from gymnastics at school?

Have you been able to attend school

regularly without interruptions caused by haemophilia?

III *Military service*

Have you been exempted from military service?

IV *Occupation*

Have you received vocational training?

Have you received special vocational training because of your haemophilia?

Can you do your work as well as a healthy person?

In what respects is your haemophilia a handicap in your occupation?

Are you able to earn your living by your occupation?

V *Social help*

Have you received social help for special schooling or vocational training?

Are you in receipt of a disablement pension?

Are you in receipt of any of the following forms of social help

apartment for disabled persons?
hand-controlled motor-car for disabled persons?
loan for starting own business?
medical care or medicine?

Do you receive blood or plasma transfusions

only as in-patient?
only as out-patient?

Are your transfusions, hospitalizations and doctors fees paid by

you?
the hospital?
the County Borough?
the County Council?
the National Health Service?
any other authority?

What form of social help would be desirable to increase your possibilities of living a "normal" life?

Incidence and Distribution of Haemophilia in Sweden

The estimated number of liveborn haemophiliacs is shown in table I. The estimate is based on the haemophiliacs born in 1931—1955. Before 1931 it is probable that many haemophiliacs were born and died without their condition being diagnosed. Consequently the number of known haemophiliacs born before 1931 is not representative. Nor is the last 5-year period (1951—1955) representative, since moderate or mild haemophilia is not always detected at an early age. As shown in an earlier publication (15) only 33 of 38 moderate haemophiliacs, i. e. 87 per cent, and 29 of 48 mild haemophiliacs, i. e., 60 per cent, had their first manifestation of haemophilia before 8 years of age. The Swedish series contains about the same number of severe haemophiliacs and of moderate and severe haemophiliacs collectively. The number born in the last 5-year period (1951—1955) i. e. 29 must therefore be corrected for the late appearance of the disease. This corrected number 34 is bracketed in table I.

Table I. Incidence of haemophilia in Sweden. Number of haemophiliacs born in 1931—1955

5 year period	No. of haemophiliacs born	No. of liveborn males	% of males born per haemophiliac born
1931—1935	31	224,810	7200
1936—1940	33	229,546	6950
1941—1945	34	312,783	9220
1946—1950	44	321,021	7300
1951—1955	29 (34)	270 725	9330 (8220)
1931—1955	173 (178)	1,379 494	7970 (7740)

The bracketed figures are corrected for the late appearance of manifestations of haemophilia (after 8 years of age).

In mild haemophilia as shown by the coagulation study on haemophilia in Sweden (9) only half of the patients had a prolonged clotting time in whole blood If these patients had not had abnormal bleeding after e g tooth extractions and major or minor surgery their disease would not have been diagnosed It is therefore highly probable that the real number of mild haemophiliacs born during a given period is higher than we have actually found Since these unrecognized mild forms of haemophilia did not require hospitalization or ambulant therapy they can however be disregarded from the medico-social point of view

As seen from table I one haemophiliac per 8000 liveborn males was born in Sweden during the years 1931—1955

The incidence and distribution by place of residence of known haemophiliacs living in Sweden in December 1960 are shown in table II The *incidence of living haemophiliacs* was 6.5 per 100 000 male inhabitants *i e.*, *1 haemophiliac per 15 000 men* or 3.3 per 100 000 inhabitants both male and female. The distribution was fairly even throughout the country although three counties, H K and S (Kalmar Blekinge and Värmlands lan) showed an appreciably higher incidence than the mean This also applied to counties A+B M and O which include the three largest cities in Sweden (Stockholm Gothenburg and Malmo) An appreciably lower incidence of haemophilia was found in counties X and Y (*Gävleborgs and* Västernorrlands lan)

Comments

The incidence of liveborn haemophiliacs was *estimated* by Ikkala (7) to be one per 6500 liveborn males in *Finland He based his estimate on the* number of haemophiliacs born during *the 5 year period 1943—1947* and assumed that the haemophiliacs born *during this period* were accurately represented in his series The corresponding value in the Swedish series is one liveborn haemophiliac per 7300 liveborn males during the 5-year period 1946—1950 The mean value for the period 1931—1955 is one liveborn haemophiliac per 8000 liveborn males However as has been pointed out previously (9) only half of the mild haemophiliacs have a prolonged coagulation time and the clinical symptoms are also mild in this group. It is therefore probable that the actual number of haemophiliacs born if all mild haemophiliacs are included is even higher than the number found for the 5-year period 1943 —1947 by Ikkala (7) or than our figure for 1946—1950 In 1943 Andreassen (1) estimated the incidence of liveborn haemophiliacs to be 1 7500 liveborn males, which is in good agreement with Ikkala s (7) series and with ours

The incidence of living haemophiliacs has been estimated in several countries. In 1945 Andreassen (1) reported one haemophiliac per 22,400 men in Denmark and in 1960 Sjolin (18) also in Denmark found one haemophiliac per 14 000 men In 1960 Ikkala (7) found the number of living haemophiliacs in his series to be one per 17 000 *male inhabitants* of Finland and estimated the total number at *13,000—14,000* men In 1954 Fonio (5) found 79 haemophiliacs *per 2,272 025* male inhabitants of Switzerland *i e.*, one per 29 000 men In 1955 den *Ottolander* (11)

Table II Distribution of living haemophiliacs in Sweden on December 31 1960

County		No. of inhabitants			No. of living haemophiliacs				No. of living haemophiliacs per	
Letter	Name	Male	Female	Total	Severe form	Moderate form	Mild form	Total	100,000 males	100,000 inhabitants
A	Stockholms stad	377,563	429,340	806,903	14	8	11	33	8.8	4.1
B	Stockholms län	231,274	231,644	462,918	8	1	5	14	6.1	3.0
C	Uppsala län	82,780	85,076	167,856	—	3	—	3	3.6	1.8
D	Södermanlands län	114,560	113,364	227,924	3	—	1	4	3.5	1.8
E	Östergötlands län	178,288	179,497	357,785	2	5	3	10	5.6	2.8
F	Jönköpings län	143,263	142,138	285,401	7	—	—	7	4.9	2.5
G	Kronobergs län	81,410	77,684	159,094	—	3	1	4	4.9	2.5
H	Kalmar län	119,073	116,562	235,635	6	3	12	21	17.6	8.9
I	Gotlands län	27,337	26,872	54,209	1	—	—	1	3.7	1.8
K	Blekinge län	72,728	71,770	144,498	4	2	4	10	13.8	6.9
L	Kristianstads län	129,071	127,477	256,548	4	—	—	4	3.1	1.6
M	Malmöhus län	308,576	319,540	628,116	9	8	13	30	9.8	4.7
N	Hallands län	85,841	84,170	170,011	2	1	1	4	4.7	2.4
O	Göteborgs & Bohus län	309,052	316,314	625,366	13	7	6	26	8.4	4.2
P	Älvsborgs län	187,153	187,884	375,037	1	5	4	10	5.3	2.7
R	Skaraborgs län	126,737	123,164	249,901	3	—	5	8	6.3	3.2
S	Värmlands län	147,786	143,220	291,006	11	—	3	14	9.5	4.8
T	Örebro län	131,621	130,913	262,534	4	—	—	4	3.0	1.5
U	Västmanlands län	119,227	113,739	232,966	1	3	3	7	5.9	3.0
W	Kopparbergs län	145,268	141,041	286,309	6	—	3	9	6.2	3.1
X	Gävleborgs län	148,202	145,139	293,341	1	1	1	3	2.0	1.0
Y	Västernorrlands län	144,484	141,261	285,745	1		—	1	0.7	0.4
Z	Jämtlands län	72,033	67,885	139,918	—	—	4	4	5.6	2.9
AC	Västerbottens län	123,169	116,535	239,704	3	1	5	9	7.3	3.8
BD	Norrbottens län	135,106	126,832	261,938	6	—	1	7	5.2	2.7
	Total: Sweden	3,739,812	3,755,958	7,495,770	107	51	89	247	6.6	3.3
	Living abroad				6	—	—	6		

estimated the number of haemophiliacs in the Netherlands at 1000 i e one per 10 000 inhabitants Tuyns (21) in 1959 found one haemophiliac in 65 000 inhabitants in Belgium In Sweden Skold (19) in 1944 found 101 haemophiliacs per 3,240 631 living males, i e one in 32,000 Our series shows an incidence of one haemophiliac per 15 000 male inhabitants in Sweden in good agreement with the figures of Ikkala (7) and Sjolin (18) As already pointed out the actual number of haemophiliacs is probably greater if all mild forms are included A rough estimate of the actual number of living haemophiliacs can be made in the following way In a previous study the number of living mild haemophiliacs was found to be 89 (Ref 16 table IV) Presuming that only half of the mild haemophiliacs have been detected their actual number would be 178, giving a total of 342 haemophiliacs and an incidence of one in 11 000 men Moreover the incidence of living haemophiliacs is steadily increasing owing to the higher mean age at death (see next section)

Table III Deaths from haemophilia in Sweden, Number of haemophiliacs belonging t families 1—180

Haemophilia Clinical form	Died before 1900	Period I 1900—1942	Period II 1943—1957	Period III 1958—1960	Total
Severe	37	81	50	3	171
Moderate	3	16	5	1	23
Mild	8	22	15	3	48
Total	48	119	70	7	244

Causes of and Mean Age at Death

Totally 244 haemophiliacs belonging to families 1—180 had died before 1961 The number of deaths in each of the three aforementioned periods is shown in table III It is seen that 119 haemophiliacs died during period I (1900—1942) and 70 during period II (1943—1947) The cause of death and age at death in the clinically severe moderate and mild cases of haemophilia during periods I and II are shown in tables IV—VI

In severe haemophilia (table IV) the main causes of death during period I were haemorrhage from internal trauma (12 cases) and spontaneous gastrointestinal (11 cases) cerebral (11 cases) and other internal haemorrhage (10 cases) The largest group was bleeding without any definite localization (18 cases) this group includes many cases in which the cause of death was given as "haemorrhage" without further notation of the cause or site. During period II the main causes of death were cerebral haemorrhage (12 cases) gastrointestinal (9 cases) renal and other internal haemorrhage (5 cases each) and bleeding without definite localization (7 cases) The mean age at death was 16.5 years in period I (1900—1942) and 23 2 years in period II (1943—1957) i e a prolongation of about 50 per cent in the latter period A comparison between the causes of death shows a decrease in the latter period of death due to traumatic haemorrhage internal haemorrhage and bleeding without any definite localization No death from nasal haemorrhage occurred in period II During this period there wa a slight proportional increase in deaths from postoperative gastroin

Table IV Causes of death and age at death severe haemophilia

Cause of death	Period I 1900–1942 Age at death, yrs									Period II 1943–1957 Age at death, yrs								
	0–5	6–10	11–15	16–20	21–30	31–40	41–60	> 60	Total	0–5	6–10	11–15	16–20	21–30	31–40	41–60	> 60	Total
Haemorrhage																		
External trauma	2	—	—	—	—	—	—	—	2	—	—	—	—	1	—	—	—	1
Internal trauma	8	1	1	—	—	1	—	—	12	2	—	—	—	1	—	—	—	3
Postoperative	1	2	—	3	—	1	—	—	7	1	—	1	1	—	—	1	—	4
Nasal	—	1	—	—	1	—	—	—	2	—	—	—	—	—	—	—	—	—
Gastrointestinal	—	1	—	1	4	2	3	—	11	1	3	—		2	1	2	1	9
Renal	—	1	—	—	—	2	—	—	3	1	—	—	—	1	—	3	—	5
Cerebral	4	1	3	—	2	—	1	—	11	5	1	—	1	1	1	3	—	12
Other internal	2	3	—	—	4	1	1	—	10	—	—	1	1	1	2	—	—	5
No definite localization.	10	—	3	—	3	2	—	—	18	2	1	1	1	1	1	—	—	7
Other causes	1	1	—	1	1	—	1	—	5	—	1	—	—	1	1	1	—	4
Total	29	10	7	5	15	9	6	—	81	12	6	3	4	9	6	10	1	50
Mean age at death, yrs					16.5									23.2				

Table V Causes of death and age at death moderate haemophilia

Cause of death	Period I 1900–1942 Age at death, yrs									Period II 1943–1957 Age at death, yrs								
	0–5	6–10	11–15	16–20	21–30	31–40	41–60	> 60	Total	0–5	6–10	11–15	16–20	21–30	31–40	41–60	> 60	Total
Haemorrhage																		
External trauma	—	—		—	—	—	—	—		—	—	—	—	—	—	—	—	—
Internal trauma	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Postoperative	—	—	—	—	2	1	—	—	3	—	—	—	—	—	—	—	—	—
Nasal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gastrointestinal	2	—	—	—	—	1	—	—	3	—	—	—	1	1	—	—	—	2
Renal	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—
Cerebral	—	—		—	—	—	1	—	1	—	—	—	—	—	—	2	—	2
Other internal	—	—	1	1	1	—	—	—	3	—	—	—	—	—	—	—	—	—
No definite localization	1	2		2	—	—	—	—	5	—	—	—	—	—	—	—	—	—
Other causes	—	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	3	2	1	3	3	2	2	—	16	—	—	—	1	1	—	3	—	
Mean age at death, yrs					19.9									40.1				

estimated the number of haemophi liacs in the Netherlands at 1000 *i e* one per 10 000 inhabitants Tuyns (21) in 1959 found one haemophiliac in 65 000 inhabitants in Belgium In Sweden Skold (19) in 1944 found 101 haemophiliacs per 3,240 631 living males *i e.*, one in 32 000 Our series shows an incidence of one haemophi liac per 15 000 male inhabitants in Sweden in good agreement with the figures of Ikkala (7) and Sjolin (18) As already pointed out the actual number of haemophiliacs is probably greater if all mild forms are in cluded A rough estimate of the ac tual number of living haemophiliacs can be made in the following way In a previous study the number of liv ing mild haemophiliacs was found to be 89 (Ref 16 table IV) Presuming that only half of the mild haemophi liacs have been detected their actual number would be 178 giving a total of 342 haemophiliacs and an inci dence of one in 11 000 men Moreover the incidence of living haemophiliacs is steadily increasing owing to the higher mean age at death (see next section)

Causes of and Mean Age at Death

Totally 244 haemophiliacs belong ing to families 1—180 had died be- fore 1961 The number of deaths in each of the three aforementioned pe riods is shown in table III It is seen that 119 haemophiliacs died during period I (1900—1942) and 70 during *period II (1943—1947)* The cause of death and age at death in the clinical ly severe moderate and mild cases *of* haemophilia during periods I and II are shown in tables IV—VI

Table III Deaths from haemophilia in Sweden Number of haemophiliacs belonging to families 1—180

Haemophilia Clinical form	Died before 1900	Period I 1900—1942	Period II 1943—1957	Period III 1958—1960	Total
Severe	37	81	50	3	171
Moderate	3	16	5	1	25
Mild	8	22	15	3	48
Total	48	119	70	7	244

In severe haemophilia (table IV) the main causes of *death during* period I were haemorrhage from internal trauma (12 cases) and spon taneous gastrointestinal (11 cases) cerebral *(11 cases) and* other inter nal haemorrhage (10 cases) The largest group was bleeding without any definite localization (18 cases) this group includes many cases in which the cause of death was given as "haemorrhage" without further notation of the cause or site During period II the main causes of death were cerebral haemorrhage (12 cases) gastrointestinal (9 cases) renal and other internal haemorrhage (5 cases each) and bleeding without definite localization (7 cases) The mean age at death was 16.5 years in period I (1900—1942) and 23.2 years in period II (1943—1957) *i e* a pro- longation of about 50 per cent in the latter period A comparison between the causes of death shows a decrease in the latter period of death *due to traumatic haemorrhage internal* hae- morrhage and bleeding without any definite localization No death from nasal haemorrhage occurred in pe- *riod II* During this period there was *a slight* proportional increase in deaths from postoperative gastroin

Table VII Total number of haemophiliacs born, dead and alive during 10-year periods from 1901—1960

Haemophiliacs	1901–1910	1911–1920	1921–1930	1931–1940	1941–1950	1951–1960
Alive at end of each period	110	146	178	205	237	253
Born during each period	57	63	55	66	78	58
Died during each period	22	30	30	33	48	34
Excess of births	35	33	25	33	30	24
Mean age at death, yrs	19	12	16	28	28	34

they died of gastrointestinal, internal traumatic and internal haemorrhage at 36, 29 and 21 years of age, respectively One moderate haemophiliac died of uraemia at 56 years of age Three mild haemophiliacs died two of cerebral haemorrhage (the first of a ruptured arterial aneurysm) and one of postoperative haemorrhage, at 16, 67 and 54 years of age, respectively

Table VII shows the total number f haemophiliacs born, alive and dead during the last six decades. The number of births and deaths during these periods is fairly constant.

The death risk for severe haemophiliacs during the first 10 years of life is shown in table VIII. There was no differ nce between the death risk for severe haemophiliacs born in 1931—1940 and those born in 1941—1950 Compared with the death risk for normal boys born in 1941—1945 as given in the official Swedish statistics (4.9 per cent for the first 10 years of life) that for the severe haemophiliacs was 27 1 and 24.2 per cent respectively It was thus about 5 times as high as that for normal boys born in the middle of the last period If the total death risk from the 2nd up to the 10th year for severe haemophiliacs and for normal boys is compared, the death risk was about 10 times as high for the former

These figures are in good agreement with those of Sköld (19) considering that his series of 60 families contained at least 7 families with moderate and 4 families with mild haemophilia Sköld (19) found a 6.8 times increased death risk for haemophiliacs from the 2nd to the 11th year of life as compared to normal persons belonging to the same age group

Comments

In the recent Scandinavian surveys of haemophilia by Ikkala (7) and Sjolin (18) the average life expectancy as a whole, based on haemophiliacs dead before 1940 was only 17 and 18 years, respectively Sköld (19) in his Swedish series, found the death risk to be between 4.9 and 7.5 times greater among haemophiliacs than normal persons during the first three decades of life

The mean age at death of severe haemophiliacs, 16.5 years, during the first period (1900—1942) is in good conformity with the results of Ikkala (7) and with Sj lin s (18) report based on Andreassen s (1) figures The mean age at death in moderate or mild haemophilia during the same period 19.9 and 29 0 years, respectively is lower than Ikkala s figures.

A comparision between the two periods (1900—1942 and 1943—1957) in the present series shows a marked

Table VI Causes of death and age at death mild haemophilia

Cause of death	Period I: 1900—1942 Age at death yrs									Period II 1943—1957 Age at death, yrs								
	0—5	6—10	11—15	16—20	21—30	31—40	41—60	> 60	Total	0—5	6—10	11—15	16—20	21—30	31—40	41—60	> 60	Total
Haemorrhage																		
External trauma	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	1	1
Internal trauma	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Postoperative	—	1	—	—	2	1	—	1	5	—	—	—	—	—	1	—	—	1
Nasal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gastrointestinal	—	—	—	—	1	—	1	—	2	—	—	—	—	—	1	1	3	5
Renal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cerebral	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Other internal	—	—	—	—	2	—	—	—	2	—	—	—	—	—	—	—	—	—
No definite localization	2	2	1	—	2	—	—	2	9	1	1	—	—	—	—	2	—	4
Other causes	—	—	—	—	—	1	—	1	2	—	—	—	—	—	—	2	1	3
Total	3	3	1	1	7	2	1	4	22	1	1	—	—	—	2	6	5	15
Mean age at death, yrs	29.6									50.0								

testinal and *cerebral* haemorrhage and from other causes

In moderate haemophilia (table V) the most common causes of death *during period I* were *postoperative*, gastrointestinal and other internal haemorrhage (3 cases each) and bleeding without definite localization (5 *cases*) *During period II there was* one death from internal trauma and two each from gastrointestinal and cerebral haemorrhage The mean age *at death* was *19 9 years in period I* and 40 1 years in period II *i e* a twofold prolongation When the *causes of death in periods I and II* are compared the same tendency is found as in severe haemophilia to a higher proportion of deaths *from cerebral and gastrointestinal haemorrhage* Although the total number of deaths of moderate haemophiliacs (21 cases) *is small compared to that of* severe haemophiliacs (131 cases) the same trend to alteration in the *principal causes of death is obvious*

In mild haemophilia (table VI) the most common causes of death *during period I* were postoperative (5 cases) gastrointestinal and other internal haemorrhage (2 cases each) and bleeding without definite locali*zation (9 cases) In period II* the most common causes were gastrointestinal haemorrhage (5 cases) and *bleeding* without any definite localization (4 cases) The mean age at death was 29 6 years in period I and 50 0 years in period II *i e*, a pro*longation of about* 60 per cent A comparison between the causes of death shows an increase in deaths *from gastrointestinal haemorrhage in* the later period

During the years 1958—1960 seven haemophiliacs belonging *to the 180 families* were *reported* to have died Three were severe haemophiliacs

Table IX. Educational record

Haemophilia Clinical form	No. of patients	Pre-school age	Ordinary schools				Special education		
			Elementary	Secondary	Higher education	Total	At home	Boarding school for handicapped	Total
Severe	112	19	54	10	4	68	11	14	25
Moderate	45	6	30	4	3	37	—	2	2
Mild	78	11	61	5	1	67	—	—	—
Total	235	36	145	19	8	172	11	16	27

Table X. School attendance

Haemophilia Clinical form	No. of patients	Pre-school age	Able to follow ordinary school courses	Unable to follow ordinary school courses	Able to follow special school courses
Severe	112	19	41	52	25
Moderate	45	6	30	9	2
Mild	78	11	65	2	—
Total	235	36	136	63	27

vere haemophilia, 45 moderate and 78 mild. Altogether 36 had not reached school age, *i. e.*, were less than 7 years old.

The educational standard reached is shown in table IX. The distribution by elementary school, secondary school and further higher education is about the same as in the ordinary Swedish population. Of the 93 severe haemophiliacs 25 *i. e.* 27 per cent, received or had received special education, 11 in the home and 14 in a special boarding school for handicapped children (Eugeniahemmet in Stockholm) Of the 39 moderate haemophiliacs, only two had received such education.

Of the 68 severe haemophiliacs attending ordinary schools, 41 (60 per cent) could follow the school courses (table X) The other 27 (40 per cent) could not follow the courses, because of frequent long absences due to bleeding manifestations These haemophiliacs would have had better success if they had received special education. Of the 25 severe haemophiliacs receiving special education, 11 were taught in the home. Since this was, as a rule, confined to four hours instruction a week, the results were not comparable with those of the 14 who were in the boarding school for handicapped children. Altogether only 55 of 93 severe haemophiliacs, *i. e.* 59 per cent, received an adequate education. The other 38 (41 per cent) were prevented by their illness from obtaining adequate schooling.

Table VIII Death risk in severe haemophilia during the first 10 years of life

Year of life	Severe haemophiliacs Born 1931–1940					Born 1941–1950					Normal boys Born 1941–1945		
	No. of cases observed	Deaths	Annual death risk	Total death risk From birth	Total death risk From 2nd year	No. of cases observed	Deaths	Annual death risk	Total death risk From birth	Total death risk From 2nd year	Annual death risk	Total death risk From birth	Total death risk From 2nd year
1st	37	3	8.1	—	—	41	3	7.3	—	—	3.415	—	—
2nd	34	1	2.9	11 0	2.9	35	2	5.3	12.6	5.3	0.375	3.793	0.378
3rd	33	1	3.0	14 0	5 9	36	1	2.8	15 4	8.1	0.231	4.024	0.600
4th	32	1	3.1	17 1	9 0	35	1	2.9	18.3	11.0	0.189	4.213	0.798
5th	31	—	—	17 1	9 0	34	—	—	18.3	11.0	0.158	4.371	0.936
6th	31	1	3.2	20.3	12.2	34	—	—	18.3	11.0	0.134	4.505	1.090
7th	30	1	3.3	23.6	15.5	34	1	2.9	21.2	13.0	0.130	4.635	1.220
8th	29	1	3.5	27 1	19 0	33	—	—	21.2	13.9	0.105	4 740	1.325
9th	28	—	—	27 1	19 0	33	—	—	21.2	13.9	0.099	4.839	1 424
10th	28	—	—	27 1	19 0	33	1	3.0	24.2	16.9	0.090	4.929	1.514

prolongation of the mean age at death in haemophilia of all degrees of severity In moderate haemophilia there was a doubling of the age at death from 19 9 to 40 1 years This group was however the smallest one. In severe and mild haemophilia the increase in age at death during period II (1943—1957) was of the same magnitude.

At the time of writing, severe and moderate haemophiliacs can be estimated to have one third and one-half respectively the life expectancy of normal male inhabitants of Sweden and mild haemophiliacs to have two-thirds the normal life expectancy

With respect to the causes of death there was the same tendency to an increased proportion from intracranial and gastrointestinal haemorrhage in the material presented by Ikkala (7) and by Wilkinson *et al* (22) as in the Swedish series. However since the cases were not classified according to the clinical degree of severity during the last period in either of these investigations, no direct comparison can be made

School Attendance, Occupation and Working Capacity

Data on educational standards, school attendance occupation and working capacity were compiled for 235 of 247 haemophiliacs living in Sweden on December 31 1960 These data were based on the questionnaire sent to each living haemophiliac and on the hospital records. Although some information could be obtained from the hospital records about the 12 who failed to answer or who answered incompletely it was considered inadequate and these haemophiliacs were not included in the social survey Of the 235 included 112 had clinically se-

Table XI. Professional or vocational training

Haemophilia Clinical form	No. of patients	No. > 20 yrs old	Higher education for intellectual professions	Ordinary vocational training schools	Special vocational training for handicapped	No professional or vocational training
Severe	112	45	12	7	8	17
Moderate	46	31	7	10	4	10
Mild	78	53	6	14	—	33
Total	236	129	25	31	12	60

Table XII. Occupations and possibilities of earning their living

Haemophilia Clinical form	No. > 20 years old	Intellectual work			Business and light work			Light manual work			Heavy manual work			No work (disabled or retired)
		No.	Earning possibilities		No.	Earning possibilities		No.	Earning possibilities		No.	Earning possibilities		
			Good	Poor		Good	Poor		Good	Poor		Good	Poor	
Severe	45	3	3	—	7	6	1	21	5	16	2	—	2	13
Moderate	31	4	4	—	6	6	—	17	8	9	2	1	1	2
Mild	53	6	6	—	11	10	1	17	16	1	17	13	4	2
Total	129	13	13	—	24	22	2	55	29	26	21	14	7	16

Table XIII. Social benefits

Haemophilia Clinical form	No. of haemophiliacs > 20 years old	Exempted from military service	Disablement pension	Social benefits: Financial aid	Special apartment	Special motor car	Loan for profession	Medical care
Severe	45	45	27	6	1	5	—	1
Moderate	31	25	9	7	—	—	—	—
Mild	53	26	2	2	1	—	—	1
Total	129	96	38	15	2	5	—	2

watchmakers, opticians and craftsmen, only two (one severe and one mild haemophiliac) were not self supporting. In the group of light manual workers, e. g. light factory workers, waiters, electricians, bookbinders and printers' assistants, only 5 of 21 severe haemophiliacs, *i. e.* 24 per cent, were self-supporting. Of the 17 moderate haemophiliacs, 8 (47 per cent) could earn their living by light manual work. All but one mild haemophiliac, *i. e.* 94 per cent, replied that they had good possibilities of earning their living at this type of occupation. Neither of the two severe

Two examples can illustrate these patients situation

Case A.J., family 73 IV 22 b 1945 Haemophilia A severe form. First symptom (haemarthrosis) *at one year of age*, then repeated haemarthroses, which have produced a stiff hip joint and poor function of the ankle knee and elbow joints. He attended ordinary elementary school during the first three years and could follow the course excellently in spite of being away for long periods because of bleeding episodes. He could *not however* continue in ordinary school as his *schoolfellows did not give* him special consideration because of his haemophilia, and he therefore received many bumps and bruises, which caused bleeding The teacher arranged a course of study at home, and with four hours teaching a week the boy could follow the ordinary courses excellently However a new teacher did not take the same *interest in him and his education was not continued. The patient has no voca*tional training, and lives on a disablement pension and social benefits.

If this patient with adequate intellectual capacity had been given educational facilities, he would probably have earned his own living.

Case L.L., family 36 IV 1 b 1929 Haemophilia A, severe form. First symp*tom (subcutaneous haemorrhages)* at one year of age later repeated haemarthroses, which produced a stiff left knee joint and severely impaired function of the right knee and ankle joints. He was able to attend elementary and secondary school and followed the ordinary courses in spite of long bleeding episodes. *He completed his education and profes*sional training at night school, and is now a specialised engineer in the construction industry He is fully capable of earning his living and has a motor car without a disablement subsidy

This patient has about the *same intel*lectual *capacity and the same severe* degree of disablement as Case A.J However he obtained a good education and he now has full working capacity

Of the 39 moderate haemophiliacs of school age or over 9 (*23 per cent*) could not *follow ordinary courses*. Two of them attended the *boarding school for handicapped children* All but two of the 67 mild haemophiliacs could follow ordinary school courses.

Of the 235 haemophiliacs who had given full answers to the questionnaire, 129 were over 20 years of age Of these 45 were severe, 31 moderate and 53 mild haemophiliacs As seen from table XI 13 of the 45 severe haemophiliacs (29 per cent) had received higher education for intellectual occupations, 7 (15 per cent) had completed ordinary vocational training schools 8 (18 per cent) had finished special training schools and 17 (38 per cent) had no vocational training Of the 31 moderate haemophiliacs, 7 (23 per cent) had higher education Ten (32 per cent) had attended an ordinary vocational training school 4 (13 per cent) a special training school and 10 (32 per cent) had no vocational training Of the 53 mild haemophiliacs, 6 (11 per cent) had higher education 14 (26 per cent) had attended an ordinary vocational training school none a special training school and 33 (63 per cent) had no vocational training

Table XII shows the *occupations of* the haemophiliacs, and their ability *to earn their living* The total 13 hae*mophiliacs, of all degrees* of severity *engaged* in intellectual work, *e.g.*, en*gineers office* managers head clerks *and* journalists, had a good earning potential Of the 24 haemophiliacs in business or doing light work, such as shop assistants clerks, draughtsmen

philiacs earning a full livelihood in an intellectual profession or in business.

However the serious problems arise in those cases where the intel lectual capacity does not permit school studies and training for such occupations. As stated earlier it is of utmost importance to give the severe *haemophiliacs* a training suitable to their intellectual capacity

In the group of *moderate haemophiliacs* 30 of 39 could follow ordi nary school courses, 21 of 31 adults had completed a vocational training course and 19 could earn a liveli hood. Thus, about one-third of the moderate haemophiliacs were incapa citated for work and could not earn their living As in severe haemophilia, they were incapacitated for ordinary types of work and might ha e had better possibilities of earning their living in physically light occu pations. However one patient (A. P family 27) is still capable at 53 years of age of doing heavy manual labour

The *mild haemophiliacs* could practically all i. e 96 per cent, follow the rdinary school course and had no special vocational training. Only six were occupationally handicapped another two were over 70 years f age and had therefore retired. Four of the handicapped (ged 57 25 28 and 38 years) had done heavy manual work a farmers and lumbermen In these cases, complications following injuries suffered at work were the main cause of incapacity

Hospitalization and Blood Transfusion Therapy

An important medico-social probl m for ha mophiliacs is their frequent need of hospitalization and blood transfusion therapy One of the main aims of Sköld s (19) investiga tion of the Swedish haemophiliacs in 1942—1943 was to put them in contact with regional hospitals where blood transfusions could be made available

The present investigation was started as a follow-up of the results of Sköld's work. The effectiveness of his work is reflected in the increase in mean age at death and the change in the principal causes of death, which have already been discussed. The extent to which hospitalization and blood transfusion therapy were needed by the haemophiliacs during the years 1943—1957 is shown in table XIV The figures were calcu lated from the available hospital records and from the questionnaires returned by the haemophiliacs, and are thus minimum values. Table XIV shows that the total length of hospi talization for the living haemophiliacs at the end of the period was 2605 weeks (approximately 10,250 days) and the total number of blood transfusions was 9362.

The comparison between the se vere, moderate and mild haemophi liacs included in table XIV shows that the *mean duration of hospitalization* for each year and case was 1.0 0 7 and 0.3 week, respectively The corresponding number of blood transfusions for each case per year was 3 9 1.6 and 1.2, respectively In the *group with severe haemophilia* the length of hospitalization and the number of blood transfusions increased with rising age of the pa tients thus the five patients in the oldest group, born in 1910 and earlier each spent about one year in hospital during the 15 years (1943—1957)

haemophiliacs doing heavy manual work both farmers was self supporting Of the two moderate haemophiliacs doing heavy manual work one (a construction worker) could earn his living but the other (a lorry driver s assistant) could not In the group of mild haemophiliacs, 13 of 17 *i e., 76 per cent could earn their* living by heavy labour

Out of 129 haemophiliacs, a total 16 (12 severe, 2 moderate and 2 mild) *i e.,* 12 per cent, had no occu*pation and lived solely* on their disablement pension and social benefits. As seen from table XIII 38 haemophiliacs (27 severe, 9 *moderate* and 2 mild) *i e* 20 per cent, had a disablement pension Twenty two of them (15 severe and 7 moderate haemophiliacs) had an occupation and worked *sporadically but were not able to* exist on their earnings.

Social benefits were of different kinds. Thus 16 haemophiliacs (6 severe 7 moderate and 2 mild) had fi*nancial aid from the County* Borough two (one with severe and one with mild haemophilia) had special apartments for disabled persons Five severe haemophiliacs had a special *hand-controlled motor car* which was also tax free Two (one severe and one mild haemophiliac) had spe*cial* sickness benefits for medical treatment All the haemophiliacs had the ordinary National *Health Insurance* with free hospital care and reduced fees for out patient treatment.

Comments

The clinical features of haemophilia have social consequences at an early age Beginning at school age, there are difficulties *for* severe haemophiliacs in particular Favre—Gilly (3 4) stated that of 20 adult haemophiliacs only 9 had been able to attend school with fair regularity the remaining 11 had *been absent from school* an average of one-third of the time Only two of the 20 were able to continue their studies through college level *and* only six others completed their se*condary school studies* The school room and especially the school play ground present a constant threat of injuries leading to haematomas or haemarthrosis

In the *present series, 41 of 93 severe haemophiliacs* could follow ordinary school courses the same proportion as in *Favre—Gilly's (3 4)* series Ikkala (7) had 15 severe haemophiliacs of secondary school age in his Finnish series of whom only 4 continued their studies only 30 per cent *of all the adult haemophiliacs* had vocational training and about a third of the severe haemophiliacs could earn their living In the Swedish series, 31 of 45 severe haemophiliacs, *i e.,* 69 per cent could not earn their living the same proportion as that found by Ikkala (7) Of the severe Swedish haemophiliacs, only 17 of 45 had no vocational training *i e* 38 per cent compared with 70 per cent in the Finnish material Despite the higher proportion with training in the Swedish group, the possibilities of earning a livelihood were not better *Ikkala (7) regarded* the lack of suitable training as the principal reason for incapacity to work in the majority of his cases. At least one important cause of unemployability is *the choice of an unsuitable* vocation by the severe haemophiliacs, as also *pointed out by Favre—Gilly (3 4)* In the Swedish *series* there are several examples of severe *haemo-*

philiacs earning a full livelihood in an int llectual profession or in business

However the serious problems arise in those cases where the intellectual capacity does not permit school studies and training for such occupations. As stated earlier it is of utmost importance to give the severe haemophiliacs a training suitable to their intellectual capacity

In the group of *moderate haemophiliacs* 30 of 39 could follow ordi nary school courses, 21 of 31 adults had completed a vocational training course and 19 could earn a livelihood Thus, about one-third of the moderate haemophiliacs were incapa citated *to work and could not earn* their living As in severe haemophi lia, they were incapacitated for ordi nary types of work and might have had better possibilities f earning their living in physically light occupations. However one patient (A. P family 27) is still capable at 53 years of age of doing heavy manual labour

The mild haemophiliacs could prac tically all *i e* 96 per cent, follow the ordinary school course, and had no special vocational training. Only six were occupationally handicapped another two were over 70 years of age and had therefore retired. Four of the handicapped (aged 57 25 28 and 38 years) had done heavy manual work as farmers and lumbermen. In these cases, complications following injuries suffered at work were the main cause of incapacity

Hospitalization and Blood Transfusion Therapy

An important medico-social problem for haemophiliacs is their frequent need of hospitalization and blood transfusion therapy One of the main aims of Sköld's (19) investigation of the Swedish haemophiliacs in 1942—1943 was to put them in con tact with regional hospitals where blood transfusions could be made available.

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The comparison between the severe, moderate and mild haemophiliacs included in table XIV shows that the mean duration of hospitalization for each year and case was 1.0 0 7 and 0.3 week respectively The corresponding number of blood transfusions for each case per year was 3.9 1.6 and 1.2 respectively In the group with *severe haemophilia* the length of hospitalization and the number of blood transfusions increased with rising age of the patients thus the five patients in the oldest group, born in 1910 and earlier each spent about one year in hospital during the 15 years (1943—1957)

Table XII Hospitalization (weeks) and blood transfusions (400-ml units) Period II 1943—1957

Haemophilia Clinical form	Born	No. of cases	Hospitalization		Blood transfusions		Hospitalization Weeks/year and case	Blood transfusions Units/year and case
			Weeks	Weeks/cases	Total units	Units/cases		
Severe	Before 1910	5	259	51.8	933	186.6	3.5	12.5
	1911—1920	8	192	24.0	1185	148.1	1.6	9.9
	1921—1930	*11*	258	23.2	1246	113.3	1.5	7.6
	1931—1940	18	316	17.6	1142	63.4	1.2	4.2
	1941—1950	37	573	15.5	1762	47.6	1.0	3.2
	1951—1957	34	142	4.2	350	10.3	0.5	1.1
Severe	Total	113	1739	15.5	6618	58.6	1.0	3.9
Moderate	Before 1910	7	31	4.4	57	8.1	0.3	0.5
	1911—1920	8	56	7.0	223	29.2	0.5	1.9
	1921—1930	9	213	23.7	716	79.5	1.6	5.3
	1931—1940	9	26	2.9	17	1.9	0.2	0.1
	1941—1950	13	120	9.2	152	11.7	0.6	0.8
	1951—1957	5	62	12.4	35	7.0	1.3	0.7
Moderate	Total	51	508	10.0	1199	23.5	0.7	1.6
Mild	Before 1910	17	104	6.1	669	39.4	0.4	2.6
	1911—1920	13	31	2.4	378	29.1	0.2	1.9
	1921—1930	17	90	5.3	181	10.6	0.4	0.7
	1931—1940	*13*	90	6.9	236	18.2	0.5	1.2
	1941—1950	18	32	1.8	72	4.0	0.1	0.3
	1951—1957	11	14	1.3	9	0.8	0.1	0.1
Mild	Total	89	361	4.1	1545	17.4	0.3	1.2
Total		253	2608	10.3	9362	37.0	0.69	2.47

and received about 190 blood transfusions. In *moderate haemophilia* the age group born in *1921—1930* nine cases, had the longest hospital stay and received the most blood transfusions. The values were comparable with those for severe haemophilia and this group did in fact consist almost entirely of cases with low *borderline* values of AHF or B factor (9) showing clinical features of severe haemophilia The other groups of moderate haemophilia had values for duration of hospitalization and number of blood transfusions similar to those in the mild haemophilia group

Mild haemophiliacs in the four oldest age groups born in 1940 or earlier had *approximately* the same annual hospitalization time but required an increased number of blood transfusions with rising age The two youngest groups had very low *figures* for hospitalization time and number of blood transfusions

Table XV. Out-patient transfusions to haemophiliacs at S:t Eriks hospital in 1957—1960

Case			Year							
Family no.	Haemophilia Type	Initials	1957		1958		1959		1960	
			Fresh whole blood	Fresh plasma	Fresh whole blood	Fresh plasma	Fresh whole blood	Fresh plasma	Fresh whole blood	Fresh plasma
21	A severe	C. F	5	—	1	2	3	3	1	2
27	A mod.	A. P	2	—	3	—	—	—	—	3
27	A mod.	M. H	—	—	2	—	2	3	—	8
28	A severe	S. W	4	—	7	2	8	—	6	20
36	A severe	L. L.	3	—	8	1	12	2	3	6
40	A severe	E. R.	1	—	3	—	1	—	1	—
Total			15	—	24	5	26	8	11	39
Other single transfusions to haemophiliacs			3	—	13	5	12	15	2	10
Total			18	—	37	10	38	23	13	49

As a rule blood transfusions are administered only to patients in hospital. In order to facilitate matters for haemophiliacs in Stockholm, out-patient transfusions have been administered during the last four years (1957—1960) at the blood transfusion centre at S:t Erik's hospital. Both fresh blood and plasma have been given. The time between withdrawal and administration of blood was usually less than two hours. As seen from table XV six patients have been treated with blood or plasma transfusions for at least three years. There is a steady increase in the proportion of plasma given as compared to whole blood. Our experience of this out-patient transfusion therapy is highly promising. In several bleeding episodes, a plasma transfusion could be administered within a few hours of the first clinical symptom of bleeding, which could thus be stopped at an early stage and hospitalization avoided.

Case L.L., family 36, b. 1929, haemophilia A, severe form, is a typical example of the results of early out-patient transfusion therapy. During the 1950—1957 period, he was hospitalized for a total of 96 days because of bleeding episodes. During the years 1958—1960, he has not been hospitalized more than 12 days and has been absent from his work only 20 days.

Case S.W., family 28, b. 1926, haemophilia A, severe form, is another successful example. During the period 1952—1958, he was hospitalized altogether 316 days and had no occupation. He was badly disabled by his bleedings. After a month of out-patient transfusion therapy he was able to begin working. He required no hospitalization in 1959 and 1960 and was absent from work only 21 days because of bleeding.

Comments

There is a more or less constant need for hospital care of the haemophiliacs. The extent to which they are hospitalized for bleeding episodes de-

pends on the hospital resource at the patient's place of residence (*i e* the distance from the nearest hospital and the facilities for transfusion of fresh blood at this hospital) Ikkala (7) in his review of haemophiliacs in Finland recorded the number of hospital admissions per year of age of the patients He found that the number of admissions to hospitals per annum was considerably greater in the younger than in the older age group the duration of hospitalization was not however stated We observed the same tendency in the Swedish series. Thus, the younger haemophiliacs had more hospital admissions per annum than did the older patients but when the duration of hospitalization — which we regard as a better measure of the medico-social aspect of hospitalization in haemophilia — was recorded we found that the older age group had a far longer duration and also required more transfusion therapy In 1961 Wilkinson *et al* (22) presented a series of 207 haemophiliacs, A and B seen at the United Manchester Hospitals during the years 1936—1960 247 of them were still alive at the end of the survey period This group is of the same size as the present series, and permits some comparisons. During the last 10 years of the Manchester survey period, these haemophiliacs spent 7324 days in hospital and received 1825 pints of blood (about 1 000 litres) The duration of hospitalization was in fair agreement with our figures specially as far as the last 4 years in the Manchester survey are concerned (3572 days spent in hospital) The number of blood transfusions was, however far less in the Manchester survey This can be partly explained by the difference between the distribution according to the degree of severity in the two series The Manchester series consisted in about equal parts of severe moderate and mild forms of haemophilia whereas the Swedish series contained as many severe cases as those of the moderate and mild forms collectively

The Carrier Problem

From the medico-social point of view the female carriers of haemophilia A and B also present problems. These problems are not of a clinical nature, since the women usually have no clinical manifestations of their carrier state However as has been shown in an earlier publication (10) the definite carriers of fertile age have a decreased AHF or B factor level constant in some instances within the upper range of mild haemophilia (less than 25 per cent of normal) Examples in the present series are cases G T., family 32 V A and V L., family 89 A P and A P., family 115 E E and S E., family 139 H E. and E. C., family 150 (10) Women with such a low AHF or B factor level have the same bleeding risk at minor or major surgery as mild haemophiliacs, and must be treated as such

The definite carriers, who have borne sons with severe haemophilia constitute a group of mothers with constant anxiety for their haemophilic children and the other family members are often neglected There may be a feeling of guilt for transmitting the illness to some of their sons and during their fertile years the carriers are constantly in fear of

giving birth to another son with haemophilia. These fears are often conveyed to the sisters of the haemophiliacs, although they are only potential carriers with a 50 per cent genetic chance of not being carriers. These findings are in agreement with those of Browne *et al.* (2) Patton *et al* (13) and Poinsard (14)

Swedish law permits abortion, with or without sterilization, in definite or potential carriers of haemophilia. The records of the psycho-social committee of the Royal Swedish Medical Board show that in 1943—1960 7 definite and 17 potential carriers belonging to families with severe or moderate haemophilia took advantage of the possibility of abortion. In 11 of these cases, sterilization was not performed. Five carriers were sterilized although they were not pregnant.

As pointed out earlier some carriers have such a low content of AHF or B factor that they can be considered as mild haemophiliacs. Fortunately the plasma AHF level rises during pregnancy so that these women have little increased bleeding tendency at abortion Furthermore, if the pregnancy goes to term delivery will not be associated with any increased bleeding tendency attributable to the low AHF level

Table XVI. Legal abortions and sterilization operations on haemophilic carriers. Eugenic indication

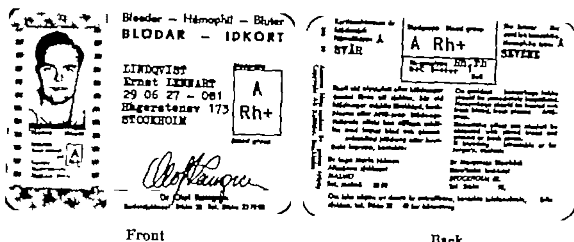
Year	Legal abortion: With sterilization	Legal abortion: Without sterilization	Sterilization without abortion
1943	—	1	—
1944	1	—	—
1945	1	—	—
1946	—	—	—
1947	—	—	—
1948	—	—	1
1949	—	—	—
1950	2	—	—
1951	1	1	1
1952	—	—	—
1953	1	2	—
1954	—	2	—
1955	—	—	—
1956	1	4	2
1957	2	1	1
1958	2	—	—
1959	1	—	—
1960	1	—	—
Total	13	11	5

By kind permission of Dr G. Hultgren, Royal Swedish Medical Board.

Discussion

From the medico-social point of view the haemophiliacs can be divided into two groups, *i. e a severe type* which contains the clinically severe and moderate forms of haemophilia, and a mild *type* which is equivalent to the clinically mild form The possibilities of a normal life differ in these two groups, as shown by tables IX—XIV The mild types of haemophiliac have far better possibilities of living a normal life haemophilia is a limiting factor only with respect to vigorous physical exercise As a rule bleeding episodes occurred in this group in our series only after accidents, in connexion with tooth extractions and major or minor surgery but when bleeding did occur these patients were as seriously threatened as those with the more severe type of haemophilia.

Our experience of the clinical and medico-social aspects of the management of haemophiliacs, especially those of the *severe type* has led us



Front Back

Fig 1 Identity card for haemophiliacs.

to adopt the programme outlined in the following discussion

Proper clinical management as well as the patient's prospects of a normal life, depend on assuring a correct *diagnosis* based on a thorough study of the coagulation system including determination of the plasma content of the factor in which they are deficient. The results of this investigation must be available to every physician who may have to care for the patient This is achieved by providing each patient with an identity card (fig 1) which gives the diagnosis blood group the essentials of therapy and the addresses of doctors who know the patient.

As early as possible the patient (or his parents) must be given full information about the disease about the symptoms likely to appear and threatening episodes of bleeding It is important for the haemophilic child to be educated up to his intellectual capacity and for him to be able to choose an occupation that does not exaggerate or provoke bleeding episodes It is also important for the haemophiliac's employer to be aware of the manifestations and nature of the disease, and for him to permit a few days absence for treatment of minor bleeding episodes, such as intramuscular haematoma or haemarthrosis.

To keep the haemophiliac fit for work requires him to be in close contact with a physician, as well as with a hospital with resources for intensive AHF plasma or blood therapy The sooner after the onset of a bleeding episode that therapy with an AHF preparation fresh or stored whole blood is started the better are the results, and the more prompt is the haemophiliac's return to work

As reported in an earlier publication (15) bleeding after dental extraction is one of the most common manifestations of moderate or mild haemophilia In severe haemophilia bleeding after tooth extraction is a severe threat Prophylactic dental care is therefore one important step in minimizing the bleeding episodes in haemophilia and if dental extractions are needed they must be carried out in hospital under the protection of AHF or plasma therapy

In view of the increased risk of

injuries and physical strain, haemophiliacs should be exempted from military service.

The family history must be penetrated, and in families with severe or moderate haemophilia, all potential carriers of fertile age should be investigated. As stated in a previous publication (10) a low AHF and B factor value, respectively can be demonstrated in definite carriers of fertile age and in about half of the potential carriers. Consequently a good chance exists of determining the definite carrier state in a potential carrier. The definite carriers must be informed of the risk of giving birth to new haemophiliacs or carriers. If pregnant, they must be allowed abortion, with or without sterilization.

The haemophiliacs — like persons with other types of inborn metabolic disorder — must be supplied with the lacking or diminished factor. Since the National Health Service in Sweden provides free substitution therapy for persons with *e. g.* Addison's disease and agammaglobulinaemia, it would be reasonable for haemophiliacs as well to receive this type of benefit. This cannot however be done under the existing legislation, because neither blood plasma nor the AHF preparation is a pharmaceutical product. The haemophiliacs receive transfusion therapy in hospital, where the beds are free of charge, or at out patient departments, where they pay a moderate fee usually 5 Swedish crowns, for a blood or plasma transfusion.

As already pointed out, prophylactic dental care is of the almost importance for avoiding dental extractions. This dental care is not included in the National Health Service, and is usually a heavy financial burden for the haemophiliacs. It would be of great help in their clinical management if they were to receive regular prophylactic dental care free of charge. Many dental extractions would thus be avoided.

In certain countries (8, 20) "haemophilia associations" have been started. The main purpose of these associations has been the organization of special services for the haemophiliacs, and the promotion of their social welfare. In a country like Sweden, with a low population density and well organized medico-social security system, there is no direct need of such an association, since most of the problems which would be in its province have already been solved in another way.

Summary

A study has been made of the medico-social aspects of haemophilia in Sweden. It is based on a survey of the hospital records and answers to questionnaires sent to 235 haemophiliacs alive on December 31, 1960.

The incidence of haemophilia is found to be one haemophiliac born per 8000 liveborn males, and one living haemophiliac per 11 000 men.

A comparison is made between two periods, *i. e.* 1900—1942 and 1943—1957 with respect to the causes of and mean age at death. It is found that the main causes of death have changed from haemorrhage after trauma and haemorrhage of unspecified localization in the earlier period to cerebral and gastrointestinal haemorrhage in the later period. In severe haemophiliacs, the mean age at death was 16.5 years in 1900—1942,

and 23 2 years in 1943—1957 The corresponding figures in moderate haemophilia were 19 9 and 40 1 years, and in mild haemophilia 29 6 and 50 0 years

Altogether 60 per cent of the severe haemophiliacs had received an adequate education one-third of them in a special boarding school for the handicapped or in their home The corresponding figures in the moderate and mild haemophiliacs were 77 and 97 per cent respectively

All but one of the severe and moderate haemophiliacs doing intellectual or light work could earn their living Totally 75 per cent of the severe and 50 per cent of the moderate haemophiliacs engaged in light manual work were disabled and could not earn their living

Altogether 27 of 45 severe haemophiliacs over 20 years old had a disablement pension this also applied to 9 of 31 with moderate haemophilia and 2 of 53 with mild haemophilia

In *1943—1957 the* severe haemophiliacs had a mean hospitalization time of one week per haemophiliac and year and received an average 3 9 *blood transfusions* The corresponding mean values in moderate and mild *haemophilia were 0 7 and 0.3 weeks* hospitalization and 1 6 and 1.2 blood transfusions

It is pointed out that from the medico-social aspect haemophilia can be divided into a severe and a mild form The mild form presents only slight medico-social problems.

Up to now no differentiation has been made between haemophilia A and B from the medico-social point of view However in view of the better possibilities of treating patients with *haemophilia A with the AHF (anti* haemophilic factor or factor VIII) preparation these two types must be evaluated separately in the future

It is emphasized that the severe form of haemophilia presents numerous medico-social problems, that must be dealt with by suitable schooling and vocational or professional training Various attempts at medico-social management of the severe form of haemophilia are discussed such as refined early diagnosis out patient transfusion therapy prophylactic dental care provision of special vocational training and choice of suitable occupations The carrier problem in severe and moderate haemophilia is also discussed

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HAEMOPHILIA IN SWEDEN

VI. Treatment of Haemophilia A with the Human Antihaemophilic Factor Preparation (Fraction I—0)

By

Inga Marie Nilsson, Margareta Blombäck and Olof Ramgren

During the last few years, much attention has been focused on the treatment of haemophilia A with concentrated antihaemophilic globulin (AHF factor VIII) prepared from human or animal plasma.

Replacement therapy of haemophilia A has largely consisted of transfusions of human blood or plasma. But in the treatment of haemophilies several situations are encountered which cannot be controlled by blood and plasma transfusions only g lesions requiring surgery and major traumatic wounds. It is now known (19 20 11) that if haemostasis is to be satisfactory the blood level of AHF must be at least 15 to 35 % of normal, and it is difficult and sometimes impossible to obtain such a level by infusion of blood and plasma. Even if the AHF in blood from blood donors lost none of its activity during collection or afterwards in the patient's circulation, it would be necessary to infuse about 3 litres of blood or 1 1/2 litres of plasma to raise the AHF blood level from zero to 35 % in an average haemophilic adult. In reality however considerable losses of AHF occur both during collection and storage of the blood and plasma (70 74 78, 79 41) The AHF is also rapidly consumed in the patient's circulation (42, 9 30 74 28) There is therefore a large demand for concentrated preparations of AHF

Many attempts have been made to prepare AHF fractions (*cf* 12) In 1937—1939 Patek, Taylor Pohle, Lozner and Kark in Boston reported

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that administration of globulin fractions, prepared in different ways from human plasma, shortened or normalized the coagulation time in haemophilic patients (67 68 69 72, 73 47 46) A globulin fraction with a similar effect had also been prepared by Bendien and van Creveld (5 6) The results reported by these earlier authors are difficult to evaluate since the methods used for assay were insensitive (coagulation time) and some of the negative results might be explained by the assumption that some of the patients had not haemophilia A but haemophilia B

Cohn s method 6 (24) for plasma fractionation was elaborated during World War II and the fractions were tested for antihaemophilic activity The Thorndike laboratory group found the activity to reside mainly in fraction I (31 88 53 44) This fraction was also given to haemophilic patients and proved to have a normalizing effect on the coagulation time. Since then fraction I has been used by several investigators, but probably because of its instability and contaminating thromboplastin prothrombin and plasminogen varying antihaemophilic activity or other discouraging results have been noted. Thus, in 1955 Winterstein as well as Koller and Deutsch expressed the opinion that the commercially available preparations are even less effective than fresh human plasma (91 92 40 20) In 1956 Aggeler Alexander and Rosenthal (3 4 80) also reported that no favourable results were obtained with fraction I Achenbach et al (2) reported Cohn s fraction I to be useful in the treatment of haemophilic patients.

In 1946 van Creveld and Mastenbroek (27) found a fibrinogen fraction prepared by the ether fractionation technique of Kekwick, Mackay and Record (38) to contain AHF In 1957 Kekwick and Wolf (39) described a modification of the method of Kekwick et al (38) A preparation was obtained which still contained fibrinogen but which had an AHF activity of 20—25 times that of human plasma per mg protein. This fraction was used with success in patients with haemophilia A (39 95, 96) Side effects were however reported

In 1955 Bidwell (7 8) described a method for purifying an antihaemophilic fraction from animal plasma, i e from bovine pig and sheep plasma, by precipitation with potassium phosphate and sodium citrate A purification of 100 to 400 times compared with plasma on a protein basis was obtained In 1957 preparations with still greater activity were reported (51) Treatment of haemophilic patients with this fraction has been described by the Oxford group (48 51 49 50 10 83 35) Good results have been obtained in vivo although side effects and antigenicity of the preparations limit its use. Macfarlane et al (51) thus recommend these preparations only for emergencies These animal AHF preparations have also been used by other authors (32 34 36) with more or less favourable results

In 1956 Blombäck and Blombäck (14) described a method for purifying fibrinogen by treating Cohn s fraction I with 1 M glycine solution containing ethanol and citrate With this procedure they obtained a fraction, I—0 in which 85—90 % of the protein consisted of fibrinogen Fraction I—0 was found to be devoid of

active plasmin and practically free from prothrombin and was therefore stable in solution. In addition to fibrinogen, fraction I—0 contained a high percentage of the AHF activity of the plasma. When this property of the fraction was recognized, it was administered to haemophilia A patients as well as to patients with von Willebrand's disease who, in addition to a prolonged bleeding time, have a low AHF level (54, 57 60 61 59 56, 58) The effect of fraction I—0 on 31 patients with haemophilia A has been described in previous papers (18, 17 62, 16) It was found that this fraction could be used with advantage for arresting haemorrhage in haemophiliacs, for preparing such patients for operations and for promoting subsequent healing It produced no demonstrable side effect.

McMillan, Diamond and Surgenor (62) have recently reported the use f an AHF rich fibrinogen fraction derived from Cohn s fraction I. This fraction is largely analogous to fraction I—0 (87) They used the fraction in 15 cases of haemophilia A, and reported its usefulness for the management of most haemorrhagic episodes in classical haemophilia.

Fraction I—0 has now been under trial since 1956 in Sweden and has been administered 818 times to 63 patients with haemophilia A This paper reports the results and conclusions derived from this further clinical experience.

Material and Methods

Preparation of human fraction I—0 — The AHF concentrate had been prepared from fresh normal plasma by methods described earlier (14 16) Fraction I was precipitated according to the method of Cohn *et al* (24) and extracted with an aqueous mixture of glycine, citrate and ethanol at pH 6.0 to 6.8. By this method, prothrombin and other contaminating proteins are removed whereas AHF and fibrinogen are left as a residue. This residue called fraction I—0 contains most of the fibrinogen of fraction I and is stable in solution.

One dose of AHF preparation was prepared from 1 400 to 1,600 ml of fresh normal citrated plasma and the yield was about 3 g of fraction I—0 which was divided into two bottles. Fraction I—0 was freeze-dried in standard 300 ml glass bottles with rubber stoppers, with 1.3—1.8 g of protein in each bottle

Solution and administration of fraction I—0 — An isotonic solution was prepared by dissolving 1.3—1.8 g of the freeze-dried protein (the content of one bottle) at room temperature in 100 ml of distilled water It was injected intravenously during the course of 10—30 minutes. Usually 1—2 bottles of fraction I—0 were given on each occasion.

Coagulation tests — All the methods used for collection and preparation of the blood samples and for determination of the different coagulation factors have been described elsewhere (63)

During treatment, the antihaemophilic activity in the patient s plasma was measured by the recalcification method on haemophilia A plasma (60 65 64) and the amount of AHF present expressed in per cent of that of a healthy person (taken as standard) whose AHF content coincided with the mean found for a group of

20 healthy subjects The plasma of the patients was assayed at dilutions of 1 10 and 1 20 before treatment and at dilutions of 1 10 1,20 1 50 and 1 100 after treatment When possible, the first samples after the injection of AHF were taken after an interval varying from 10 minutes to 2 hours

The antihaemophilic activity of fraction I—0 *in vitro* was assessed by its normalizing effect on the recalcification time of haemophilia A plasma, and was expressed in equivalents of millilitres of fresh human plasma The dilutions of the samples were prepared in physiological saline It was also checked that the activity of the preparations was not due to thromboplastin activity which would have shortened the recalcification time of normal plasma or haemophilia B plasma

Clinical Material

The clinical material consisted of 59 Swedish patients with haemophilia A which was severe in 40 moderate in 12 and mild in 7 The patients were classified according to AHF (*factor VIII*) level into three groups i e

severe haemophilia A AHF < 1 per cent of normal

Moderate haemophilia A AHF 1—4 per cent of normal

Mild haemophilia A AHF 5—25 per cent of normal

The family history the symptoms and the coagulation status of the patients are described elsewhere (63 75 76 77) with the exception of 2 patients, namely *H W., fam 181* and *J L., fam 187* The patients are referred to by the initials year of birth and the family number given in the earlier paper (63)

H W fam. 181 was a 3 year old boy (born 1958) with severe haemophilia A. Ever since the first year of life he had episodes of large subcutaneous and intramuscular haematomas and joint bleeding in the ankles, knees and elbows.

J.L., fam. 187 was a 71 year old man (born 1890) with mild haemophilia A.

The material included in addition, 4 patients outside Sweden One was an 8 year old Finnish boy with severe haemophilia A who was treated because of cerebral haemorrhage (Fam. A I 3 V—3 Ikkala (37) One was a Polish patient with severe haemophilia A who was treated in connection with head injury one in Zürich with severe haemophilia A was treated for gastrointestinal bleeding and one in Boston with clinically severe haemophilia A was treated in connection with cholecystectomy (Dr Diamond)

Case Reports and Results

From June 1956 to August 1961 fraction I—0 has been administered a total of 818 times to 63 patients with haemophilia A (Table 1) Twenty-six of these patients were treated at the General Hospital in Malmö 21 at hospitals in Stockholm 12 at their local hospitals in Sweden and 4 abroad The patients in Malmö and Stockholm were treated by us and with a few exceptions, the AHF assay was performed before and after the injection of AHF In the other patients the AHF activity was not followed during treatment.

One whole dose of fraction I—0 is as mentioned prepared from 1 400 to

Table I. Administration of fraction I—0 to patients with haemophilia A

Type of haemophilia A	No. of treated haemophiliacs	No. of treated episodes	No. of administrations
Severe	40	238	534
Moderate	12	25	233
Mild	7	10	24
Subtotal	59	273	791
Haemophiliacs outside Sweden	4	4	27
Total	63	277	818

1,600 ml of fresh normal plasma, and contains about 3 g of protein. The dose of fraction I—0 administered on each occasion in the present material is listed in Table II. It is clear from the table that half a dose or a whole dose of fraction I—0 was usually administered on each occasion. Only in 17 cases (5 of these patients were outside Malmö and Stockholm) did treatment with fraction I—0 consist of administration of a single half or whole dose. All the other patients re ceived fraction I—0 repeatedly (Table III) Table III shows that 17 of the Swedish patients received fraction I—0 on more than 10 occasions. Table IV gives a list of 11 patients, who recei ed a large number of infusions of fraction *I—0* during a fairly long period

No untoward reactions were observed in connection with the infu sions. Some patients in whom the fraction was given at a rather high infusion rate, however complained of a burning sensation in the mouth. No fever was observed. Three patients had previously developed immune antibodies to blood group antigens, but no reactions to the fraction were noted. No resistance to treatment was demonstrated in those patients who received repeated doses of fraction I—0 not even in those listed in Table IV We have not been able to demon strate any excess of circulating anti coagulant in the blood of the patients. The patients were studied for anti coagulants by the methods of Lewis, Ferguson and Arends (45) and Laurell and Nilsson (43) Two pa tients have developed hepatitis, namely *K S fam 45* and *L R C., fam 74* These 2 patients had received a large number of AHF injections (Table IV) The patient *K S fam. 45* (see case report major surgery) had dur ing this time also received 165 blood transfusions. The hepatitis in these cases was fairly mild

The patients were treated in con nection with major and minor surgi cal procedures and bleeding episodes such as joint bleeding, gastrointesti nal, retroperitoneal and renal bleeding, large haematomas, haemorrhage after tooth extraction and surgery The numbe of patients treated and the number of doses and injections of fraction I—0 administered in con nection with the various bleeding episodes are given in Table V

Case reports of the patients subjected to major or minor surgical procedures are given below To demonstrate the use of *fraction I—0* in connection with various bleeding epi sodes, a *few illustrative* examples of each type of bleeding will be described in detail.

Table II Dose of fraction I–0 given on each administration

Family No.	Case Initials	Type of haemophilia A	Dose of fraction I–0 0.25	0.5	1	1.5	2	Total no. of administrations
4	C. H N	Severe	—	1	—	—	—	1
10	R. A.	Severe	—	10	5	—	—	15
11	K. L.	Severe	—	4	2	—	—	6
13	N O H	Mild	—	3	—	—	—	3
13	G J	Mild	—	2	—	—	—	2
17	I A.	Severe	—	1	—	—	—	1
18	G B	Severe	—	22	6	—	—	28
18	N G W	Severe	—	1	—	—	—	1
22	P F	Severe	—	1	—	—	—	1
27	M H	Moderate	—	16	16	1	—	33
27	G H	Moderate	—	3	—	—	—	3
27	C A	Moderate	—	5	—	—	—	5
27	A. A.	Moderate	—	16	3	1	—	20
27	L. J	Moderate	—	13	—	—	—	13
27	B K.	Moderate	—	3	—	—	—	3
32	T S	Severe	4	20	2	—	—	26
32	U T	Severe	—	6	—	—	—	6
35	R. L.	Severe	—	1	—	—	—	1
36	L. L.	Severe	—	1	—	—	—	1
37	C. S.	Severe	—	7	1	—	—	8
37	L. C. S	Severe	—	8	2	—	—	10
42	B R.	Severe	—	1	—	—	—	1
45	K. S	Moderate	—	72	30	4	3	118
46	B S.	Severe	—	1	—	—	—	1
48	L. W	Severe	—	3	1	—	—	4
54	L. G N	Mild	—	4	—	—	—	4
57	J S.	Severe	—	2	—	—	—	2
62	B W	Severe	—	16	4	—	—	20
66	L. C.	Severe	—	2	—	—	—	2
68	G F	Severe	—	4	4	—	—	8
70	S M	Severe	—	4	2	—	—	6
72	B. B	Severe	1	86	20	—	—	107
72	L. B	Severe	—	37	3	—	—	40
74	L. R. C	Severe	—	55	—	—	—	55
75	L. T	Severe	1	12	1	—	—	14
76	L. S	Severe	—	12	—	—	—	12
77	T S	Severe	—	1	—	—	—	1
78	B E.	Severe	—	1	—	—	—	1
84	R. H	Severe	—	19	8	—	—	27
85	B. C.	Severe	—	1	—	—	—	1
86	G S.	Severe	—	2	—	—	—	2
87	R. F	Moderate	—	7	2	—	—	9
88	R. K.	Severe	—	4	5	—	—	9
90	P B.	Severe	—	1	—	—	—	1
95	B. M.	Severe	—	1	—	—	—	1

Table II (cont.)

Family No.	Case Initials	Type of haemophilia A	Dose of fraction I—0 0.25	0.5	1	1.5	2	Total no. of administrations
97	R. F	Moderate	—	14	4	—	—	18
103	U B.	Severe	—	4	—	—	—	4
106	S. O J	Severe	—	8	—	—	—	8
108	P V	Severe	—	72	5	3	—	80
111	E. N	Moderate	—	4	2	—	—	6
123	K. A.	Mild	—	4	—	—	—	4
127	A. P	Mild	—	4	3	—	—	7
138	G P	Moderate	—	4	—	—	—	4
140	T B	Severe	—	17	—	—	—	17
143	K. J	Severe	—	1	—	—	—	1
152	S. K.	Moderate	—	1	—	—	—	1
174	D. K.	Mild	—	3	—	—	—	3
181	H. W	Severe	—	4	—	—	—	4
187	J L.	Mild	—	1	—	—	—	1
Finnish patient J H.			—	1	—	—	—	1
Swiss		Zurich	—	2	2	—	—	4
Polish			—	2	2	—	—	4
USA		Boston	—	10	8	—	—	18
		Total administrations	6	648	182	9	3	848
		Total whole doses	1.5	324	182	13.5	6	497

Table III Number of episodes treated and of administrations of fraction I—0 in patients with haemophilia A

Type of haemophilia A	No. of haemophiliacs	No. of haemophiliacs											
		No. of treated episodes					No. of administrations						
		1	2-5	6-10	11-20	21-50	1-5	6-10	11-20	21-40	41-60	61-100	> 100
Severe	40	18	12	4	3	3	20	8	5	4	1	1	1
Moderate	12	5	6	1	—	—	5	2	3	1	—	—	1
Mild	7	4	3	—	—	—	6	1	—	—	—	—	—
Total	59	27	21	5	3	3	31	11	8	5	1	1	2

Table IV Repeated treatment of haemophilia A with fraction I—0

Family	Case	Year of birth	Time period Month/year	No. of administrations of fraction I—0	No. of doses of fraction I—0
18 (severe)	G B	1912	12/56— 7/60	28	17
27 (moderate)	M H.	1921	11/56— 6/61	33	25.5
27 (moderate)	A. A.	1941	12/60— 3/61	20	12.5
32 (severe)	T S	1953	8/60— 6/61	26	13
45 (moderate)	K. S	1904	7/59—10/60	118	87
62 (severe)	B W	1942	11/58— 3/61	20	12
72 (severe)	B B	1946	6/56— 6/61	107	63.25
72 (severe)	L. B	1948	9/57— 6/61	40	21.5
74 (severe)	L. R. C.	1951	12/59— 6/61	55	27.5
84 (severe)	R. H	1938	1/57— 2/61	27	17.5
108 (severe)	P V	1945	4/58— 6/61	80	45.5

Table V. The use of fraction I—0 in 63 patients with haemophilia A

Indication	No. of cases treated	No. of episodes	No. of administrations of I—0	No. of doses of I—0
Major Surgery				
Appendectomy	1	1	4	3
Laparotomy for ileus	1	1	9	7
for nephrolithiasis	2	3	61	30.5
for nephrectomy	1	1	61	52.5
Explorative laparotomy	1	1	8	4.5
Cholecystectomy	1	1	16	13
	—	8	159	110.5
Minor Surgery				
Tooth extractions	5	6	40	34
Circumcision for phimosis	1	1	3	1.5
Incision of phlegmon	1	1	17	11
Skin biopsy	2	2	3	1
Joint operations	1	5	47	33
Aspiration of joint for haemorrhage	2	2	6	4.5
Reduction of bone fracture	3	2	10	6
	—	19	144	92.0
Haemorrhage				
Haemorrhage in joints	27	83	170	88
Haematoma (spontaneous or following injury)	14	19	69	20.25
Haemorrhage in throat	2	2	8	4
Retroperitoneal haemorrhage	10	13	58	31.5
Gastrointestinal haemorrhage	13	19	64	38
Haematuria	6	9	23	14.5
Intracranial haemorrhage	6	7	30	19
Haemorrhage after:				
tooth extraction	2	2	8	5
surgery and cut wounds	3	3	12	6
aspiration of joint	1	1	8	3.25
Nose bleeding	4	4	11	5.5
Test dose	4	4	4	2
Prophylactic treatment	3	85	83	43.5
	—	250	516	285.5
Total	—	277	818	487

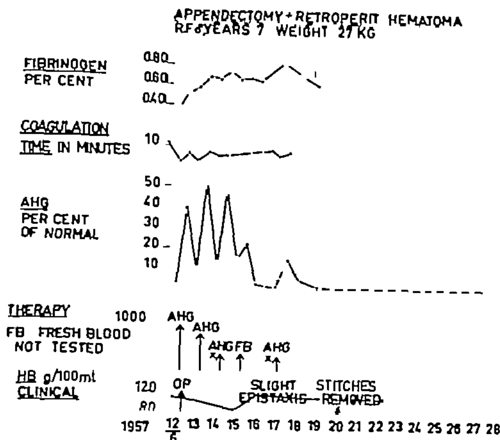


Fig 1 Treatment and course in R.F., fam 87 born 1951 The amount of AHF (=AHG) injected is expressed as the volume of normal blood with corresponding AHF activity (From Blombäck and Nilsson 1958)

Case reports

Major surgery

R.F., fam. 87 born 1949 — This was a 7 year old boy with moderate haemophilia A in whom *appendectomy* was performed under cover of AHF (Fig. 1) This case has been reported in detail in a previous paper (18 case 6) He received 4 injections of AHF (3 doses) The AHF level was kept at 40 per cent during operation and between 15 and 40 per cent during the postoperative course. The operation was performed without any increased bleeding tendency and the postoperative course was uneventful.

G.B fam 18 born 1912 — This was a 47 year old man with severe haemophilia A. In 1956 he received AHF treatment because of haematuria. This episode has been described in detail in a previous paper (18 case 3)

In February 1959 he was admitted to his local hospital (Umeå) with symptoms of ileus. A laparotomy was performed under cover of fraction I—0 on the fourth day after admission He had a roughly 8 cm long strangulated intestinal loop which seemed to be herniated but no signs of gangrene After treatment with hot saline towels the passage through the intestine was good

He received one whole dose of frac



Fig. 2. N phrolithotomy (episode I) in K.S., fam. 45 born 1904. Treatment and course.

tion I—0 before and a whole dose immediately after operation. The same day he also received 1,300 ml of fresh blood. The followi g day he received one whole dose of fraction I—0 and during the following three days half a dose each day. There was no bleeding until the tenth day after laparotomy when the stitches were removed. H then rece ed half dose and th follow g d y one wh l dose. During the follow l g 14 days the wound bled slightly n 3 occasions, nd the patie t was give a further whole dose of fraction I—0 nd 450 ml f fresh blood. H left the hospital one mo th after operation in good health.

H rece ed total of 9 injections of AHF (7 doses)

G.P. fam. 136 born 1908. — This was 51 year old man with moderate haemophili A. Ureterolithotomy was per formed at his local hospital (Gothenburg). He received 4 injections of AHF. H reco ered completely but no further deta ls have been eported t us.

K. S., fam. 45 born 1904. — This was 55 yea old man (w ight 77 kg) with moderat haemophilia A (AHF level 2 per cent, coagulation time 18 mi utes) who was subjected to three operations: *ephrolithotomy (ight side) nephrec t my and ureterolithotomy* (left side).

Since 1956 the patient had at short in tervals had right-sided coli with associated protracted haematuria. He was repeatedly admitted to hospital. Intravenous urography (June 1959) revealed stone in the right ureter. The left kidney ppeared functionally and morphologically normal, but the right kid ey appeared t be f nctionless. Since the patient had very severe pain owing to the ureteral stone, and in view of the risk of further renal stasis jeopardizing kidney fun tion, operation was considered necessary. On July 27 1959 he was subjected to *right-sided pyelolithotomy* under cover of fraction I—0 (Fig. 2). The upper ureter was exposed, but n stone could be detected. The stone had migrated up into the renal pelvis. Th i cision was therefore extended and the renal pelvis was exposed. Several roent genograms were taken during operation. Localization nd removal of th tone were difficult and the operation lasted 4 hours. The operati n was per formed without ny increased bleeding tendency. The postoperati course was on the whole smooth duri g the first 2 weeks, except that haematuria was some-

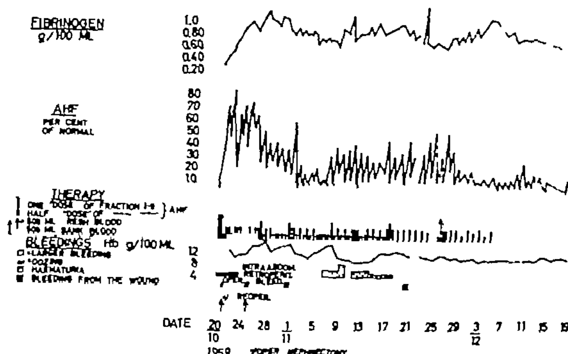


Fig. 3 Nephrectomy (episode II) in K.S. fam. 45 born 1904 Treatment and course.

what more pronounced than ordinarily after such an operation He was out of bed within 1 week. The plasma AHF was maintained at a level of 50—65 % during operation, at 30—40 % during the first 2 days after operation and then at 20—30 % during the following 8 days (Fig. 2) This was achieved in this patient by giving 2 doses of fraction I—0 before operation, one dose of fraction I—0 1 hour after operation one dose on each of the next 2 days and then half a dose 8 times during the postoperative period until the skin sutures were removed

Two weeks after the operation, by which time the urine had become clear the furthur course was complicated by obstinate and fairly severe haematuria. Repeated medication with fraction I—0 (Fig. 2) had no effect on the haematuria and the patient received 1—blood transfusions every day During - months expectant treatment the haematuria showed no tendency to disappear During this period from July 27 to October 19 1959 he received a total of 29 doses of fraction I—0 on 23 occasions.

It was then suspected that the blood in the urine might have been due to some factor other than the operative trauma and nephrectomy was considered the only possibility to control the condition. Under cover of fraction I—0 nephrectomy was done on October 1 (Fig. 3) Operation revealed a large arterial renal aneurysm which explained the haematuria (Microscopic examination of the kidney showed advanced nephrosclerosis. The aneurysm was regarded as probably due to arteriosclerosis.)

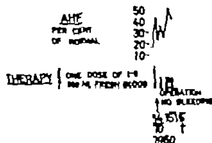


Fig. 4. Ureterolithotomy (episode III) in K.S., fam. 45, born 1904.

During the operation a d the first 2 d ys fter it the patient showed tendency to increased bleeding. He received 2 doses f fraction I—0 immediat ly before the operation, half a dose of frac tion I—0 t the end f th operation, and 5 hours after the operation a whole dose of fraction I—0 On each of the f llowing d ys he received one dose of fraction I—0. The AHF lev l was 40—80 % during and after the operation and the following days between 20—74 %. On the fourth day after the operation, the AHF fell to 20 per cent and complicating haemorrhage occurred with intraabdominal and retroperitoneal bleeding, whi h required operation. Af ter this operation the patie t general condition wa ery poor He was gi en half a dose or whole dose of AHF daily during the later course The AHF content ranged from 8 to 40 %. Owing to lack of AHF it was not possible to give such large doses of AHF as would have been necessary t keep the AHF level t 30 per cent continuously On the 15th—21st day after the last operation the patient was thus bleeding from the operation wound Transfusions of fresh blood were necessary to control the situation. From October 20 t December 20 h received a total of 52.5 doses given on 61 occasions. The patient improved subsequently and at the end of December he was home in good condition.

Half a year after the first operation the patient developed hepatitis (January 1960) from whi h he recovered within 2 months. H then felt w ll until Sept mber 25 1960 when he became ill with hills and nausea. Culture of the urine as well as of the blood was positi e for proteus. Oliguria occurred nd progressed and N.P.N increased from 62 to 222 mg/100 ml. He also had hyper kalaemia. Roentgenologic exami ation showed marked e largement f th left kidney nd a large stone in the left ureter He was severely ill. On October 14 1960 left-sided ureterolithotomy was don under cover of fracti n I—0 (*Fig. 4*) H received 2 doses of fraction I—0 before operation and on dose 8 hours after the operation. The operation was performed without any bleeding complication. The following day he received one dose each day The AHF level was kept continuously t 26 to 50 per cent. H did not show any bleeding symptoms. However the uraemic condition became worse nd bro chopneumonia developed H died on October 16 of uraemia. During this last episode he received 5 doses of AHF Post mortem examination revealed no bleeding from the operative field. He had left-sided hydronephrosis and pyelonephritis, purulent tracheobronchitis and widespread purulent focal pneumonia. The spleen showed signs of infection. H also had generalized enlargement of the heart, pronounced atheromatosis of the aorta a d coronary sclerosis.

This patient received 87 doses of fraction I—0 (118 administrations) and 165 blood transfusions

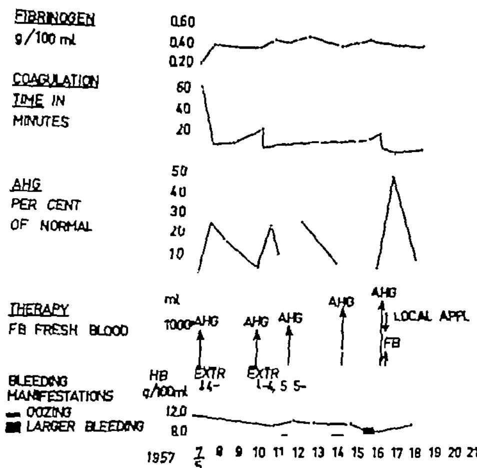


Fig. 5 Treatment and course in S.M., fam. 10 born 1950 The amount of AHF (=AHG) injected is expressed as the volume of normal blood with corresponding AHF activity (From Blombäck and Nilsson, 1958)

R.A fam 10 born 1957 — This was a 6 year old boy with severe haemophilia A In February 1959 the patient was admitted to his local hospital (Västervik) with abdominal pains. The surgeons suspected appendicitis and considered surgical exploration necessary One whole dose of AHF was given before operation. A rather large retroperitoneal haematoma was found while the appendix was normal. The abdomen was closed without removal of the appendix. The patient received 5 injections of AHF (2 whole doses and 3 half doses) in the postoperative course The postoperative course was uneventful, and he soon recovered from the retroperitoneal bleeding.

Cholecystectomy was performed on a patient with haemophilia A in Boston (Dr Diamond) under cover of fraction I—0 He received 18 administrations, and he recovered

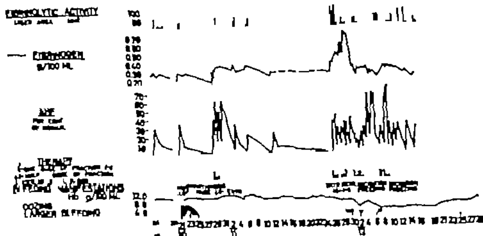


Fig. 6. Dental extractions in P.V., fam. 108, born 1945. Treatment and course.

Minor surgery

Tooth extractions

In 5 patients teeth were extracted under cover of fraction I—0

S.H fam. 70 born 1950 — A 6 year ld boy with severe haemophilia A in whom one tooth was extracted n an day and three d ys later 3 more. H recei ed 5 injections f AHF ($3^1/$ doses) Five d ys after the latter extraction profuse bleeding occurred from the socket of e of the xtracted teeth. The AHF level had then fallen t 5 per cent. After adm i tration f AHF the bleeding wa controlled. Otherwise the course was uneventful. Thi case has been reported i detail in previous paper (18 case 5) (Fig. 5)

P.V fam. 108 born 1945 — This was a 13 year old boy with severe haemophilia A. S nce early childhood he had repeatedly been admitted to hospital for joint bleedi gs nd large haematomas.

On October 29 the right lower seco d molar was extracted because of granuloma of the tip of the root (Fig. 6) One dose of AHF was given immediately before the extraction and half a dose of AHF 3 hours later During the following day he received 5 half-doses of AHF N bleeding occurred during the extraction or the postoperative course. As can be seen from the figure the AHF level was kept between 20 nd 60 per cent during the extraction nd the following 3 days.

In November he was gain admitted t hospital for extraction of the upper second molars (granuloma on roots of both teeth) On November 26 the right upper second molar was extracted. H received one dose of AHF before the extracti n nd half a dose 5 hours after The following day half a dose of AHF was gi en The AHF level was about 30—40 per cent. No bleedi g occurred.

On N vember 28 the left upper second molar was e tracted. H received half a dose of AHF one hour before. Th

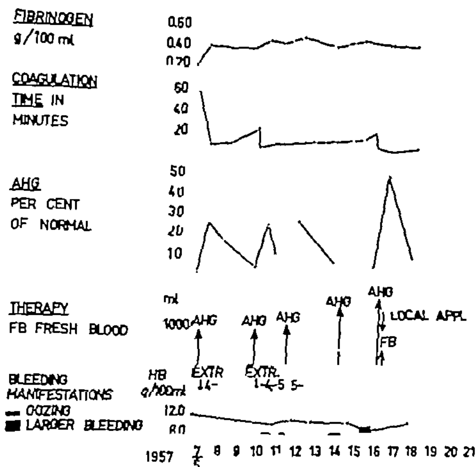


Fig. 5 Treatment and course in S.M., fam. 70 born 1950 The amount of AHF (=AHG) injected is expressed as the volume of normal blood with corresponding AHF activity (From Blombäck and Nilsson 1958)

R.A fam. 10 born 1957 — This was a 6 year old boy with severe haemophilia A In February 1959 the patient was admitted to his local hospital (Västervik) with abdominal pains. The surgeons suspected appendicitis and considered surgical exploration necessary One whole dose of AHF was given before operation. A rather large retroperitoneal haematoma was found while the appendix was normal. The abdomen was closed without removal of the appendix The patient received 5 injections of AHF (2 whole doses and 3 half doses) in the postoperative course The postoperative course was uneventful, and he soon recovered from the retroperitoneal bleeding.

Cholecystectomy was performed on a patient with haemophilia A in Boston (Dr Diamond) under cover of fraction I—0 He received 18 administrations, and he recovered

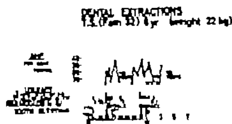


Fig. 8. Dental extractions in T.S., fam. 32, born 1953. Treatment and course

tracted, and immediately afterward he was given 400 ml of fresh plasma. The bleeding from the extraction wound did not, however stop, and he was given half dose of fraction I—0 and later in the evening a further half dose. The bleeding stopped. The following day he was given 200 ml of fresh plasma. H eacted to the injection with urticaria, but there was an effective haemostasis. After a week he left hospital healed

T.S., fam. 32 born 1953 — An 8 year ld boy with severe haemophilia A. Since early childhood he had been ad mitted to hospital for joint bleedi gs, large haematomas and haematuria (see *Sit bl pay Haemorrhage after puncture of haemarthrosis*) H had 2 teeth with granuloma of the tip of the root, and on admission he was bleeding profusely from a ruptured alveolar abscess (Fig. 8) He received half a dose of AHF a d the bleeding promptly stopped The tw premolars were then extracted, one five da fter the other He received one dose f fraction I—0 before extraction half a dose 8 hours after half dose of AHF twice the next day and then 2 and 4 half doses duri g the postoperative periods.

Circumcisio for pl imosis

P V f m. 103, born 1955. — This patient has been reported bove under *Dental xtractions.* On July 2, 1959 circumcision for phimosis was performed. He received one dose of AHF before operation. The following day h received half a dose of AHF The AHF level ra ged between 25 and 60 per cent. There was no bleeding at operation, and the postoperative course was unevent ful.

Incision of phlegmon

A.A fam. 27 born 1941 — A 20 year old boy (weight 79 kg) with moderat haemophilia A (AHF level 2 per cent) The p tient was admitted to hospital because f infected fi gers. The entire left thumb and the entire small finger on th right hand were involved by phlegmon.

H recei ed one and a half doses of AHF after which the phlegmon was in ised (Fig. 9) It proved necessary to make large incisions along th entire length of the small finger of the right hand nd vola aspect of the left thumb. The abscesses and necrotic tissue were excised During the operation the patient showed no increased tend ncy t bleed. Two hours after the operation the AHF level was 19 per cent. During the ight the patient bled from the right hand. After injection of half a dose f AHF bleeding promptly stopped. In the postoperative course he received daily i jections of fraction I—0 Th plasma AHF activity was kept between 10—40 per cent. On the fifth postoperative day the patient bled from th wound on the right hand. The bandage was soaked with blood. The haemoglobin level decreased from 13.9 g/100 ml to 10.5 g/100 ml. The AHF level had decreased to 5 per cent. After dministrati n of one dose of AHF the bleeding stopped. During the further postoperati course the patie t received 9 i jections of half-doses of AHF Some of the fractions were give as test doses and were of low cti vity The wounds healed without compli cations. H received altogether 11 doses of fraction I—0 (17 administrations)

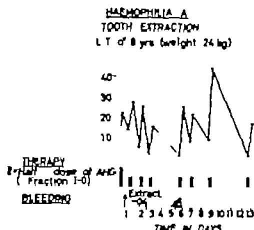


Fig. 7 Tooth extraction in L.T., fam. 75 born 1952. Treatment and course

AHF level then increased from 12 to 30 per cent Four hours after the extraction he was given half a dose and the AHF level then increased from 20 to 36 per cent The next morning he bled profusely from both the left and the right upper jaw The AHF level had fallen to 8 per cent He received one dose of AHF which increased the AHF level to 35 per cent. The bleeding stopped promptly The following morning he again began to bleed from the left jaw He received half a dose of AHF which increased the AHF level from 10 to 40 per cent. The bleeding stopped. The following morning he received one dose of AHF which proved to be inactive In the afternoon the patient started to bleed from the jaws The AHF level was then only 10 per cent. In addition the patient had developed a psychosis with considerable motor unrest and he took out the protective bridge that had been placed over the site of the extracted teeth. (He had had encephalitis and previous similar attacks of psychosis) He was given half a dose of AHF and the bleeding again stopped The following day he again started to bleed (December 2) The haemoglobin level had decreased from 11.5 g/100 ml to 7.8 g/100 ml. That day he received one and a half doses of AHF and a transfusion of bank blood which had to be discontinued because of the development of chills. The following days the AHF level was kept between 20 and 70 per cent and no bleeding occurred On December 7 the AHF level had decreased to 9 per cent, and he started to bleed again After one dose of AHF the bleeding stopped. In the further course there was no more bleeding Fig. 6 shows that the patient had an increased fibrinolytic activity in connection with the extractions and periodically in the postoperative course

L.T., fam 75 born 1952 — An 8 year old boy with severe haemophilia A. Since the age of one year he had repeatedly been admitted to hospital for joint bleedings, large haematomas and intraabdominal bleeding. He had received 5 injections of AHF during 1959—1960

In May 1961 he was admitted to hospital for extraction of a carious tooth. He received one dose of AHF and the lower left first premolar was extracted under local anaesthesia (xylocaine) A coagulum immediately formed and no bleeding occurred Five hours later half a dose of AHF was given (Fig. 7) Half a dose of AHF was also given on each of the two following days. On the fourth day he had had no bleeding, and he was sent home On the sixth day he returned to the hospital with profuse bleeding from the socket. The haemoglobin level had decreased from 15 7 g 100 ml to 1_7 g/100 ml. Administration of half a dose of AHF promptly controlled the bleeding. He received 3 further half-doses of AHF (Fig. 7) and no more bleeding occurred.

R.F., fam. 87 born 1949 — A 12 year old boy who previously had been appendectomized and had bleeding after tooth extraction (see *Major surgery* and *Bleeding after tooth extraction*)

In March 1961 he was admitted to hospital because of bleeding from the gingivae He was bleeding from the socket of a loose tooth. The tooth was ex

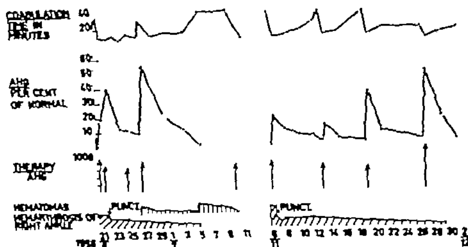


Fig. 10 Treatment and course in B.B., fam. 72, born 1948 (episodes 1 and 2) The amount of AHF (=AHG) injected is expressed as the volume of normal blood with corresponding AHF activity The clotted arrow indicates fraction I—0 with unknown AHF activity (From Blombäck and Nilsson, 1958)

The haematoma was then incised and about 500 ml of partly coagulated blood was expressed. It was not possible to remove all the blood at once On the following days the patient received AHF and blood transfusions and further amounts of blood were expressed. Treatment was not complicated by haemorrhage. He soon felt better and the knee healed well. He had received a total of 6½ doses (11 administrations) of AHF. He left hospital 16 days after admission.

The prophylactic treatment was resumed and he has not had any such severe joint bleeding since.

Aspiration of joint haematoma

L.B. fam. 72, born 1948 — A 9 year old boy with severe haemophilia A, in whom joint haematoma was punctured under cover of AHF This case has been reported in detail in a previous paper (18 case 8) Four injections of AHF (2½ doses) were given.

K.L., fam. 11 born 1944 — A 17 year old boy with severe haemophilia A. A large haematoma in the knee joint was punctured under cover of 2 whole doses of AHF No bleeding occurred.

Reduction of bone fracture

L.R.G., fam. 74 born 1951 — A 10 year old boy with severe haemophilia A. Since 1959 he had received doses of AHF on various occasions because of bleeding (see *Intracranial haemorrhage Joint bleeding Haematuria)* On May 6, 1961 he had a cycle accident and

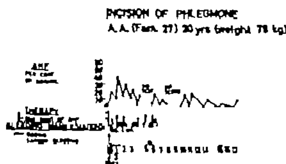


Fig. 9 Incision of phlegmone in A.A., fam. 27 born 1941 Treatment and course

Skin biopsy

U T fam 32 born 1957 and T.S., fam. 32 born 1953 — These patients had severe haemophilia. They received half a dose of fraction I—0 before removal *of a fragment of the skin for tissue culture* and chromosomal studies. No bleeding occurred

Incision or aspiration of joint haematomas

AHF was given to 3 haemophiliacs on 7 episodes in connection with incision or aspiration of joint haematomas

B.B fam 72 born 1946 — This patient had severe haemophilia A and had been treated with AHF since 1956 (see *Prophylactic treatment*) In a previous paper (18 case 1) we described 3 episodes with severe bleeding of the right ankle and the right knee (Figs. 10 and 11) The joints were markedly swollen dark blue-black and showed necrotic ulcerations. The haematomas were incised under cover of AHF The patient *received 20 injections of AHF (14 doses)* in connection with these episodes.

Since then the patient has had two further episodes of severe bleeding in the knees, and on both occasions the haematomas were incised *under cover of* AHF

Episode 4 In February 1958 the boy was admitted to hospital because of haemorrhage of the left knee which had swollen to the size of a football. The skin was blueblack and incipient necrosis was seen over the patella. The haemoglobin value was 7 4 g/100 ml, temperature 38—40 C. He had very severe pain. He was treated with AHF (Fig. 12) red blood cell suspensions and erythromycin After 8 days the haematoma was incised About 500 ml of a brownish, bloody mass was evacuated *after which* the pain abated and the body temperature began to fall towards normal. No further bleeding occurred. Later a fist-sized necrotic mass was removed from the lesion over the patella. In the floor of the cavity the tibia and the fibula lay bare The muscles around the knee joint had atrophied completely During the later course treatment was limited to half a dose of AHF once or twice a week. The cavity healed satisfactorily without complicating hæmorrhage In connection with this episode (Fig. 12) the patient received 12½ doses (16 administrations) of fraction I—0

After this bleeding period the patient was given a prophylactic dose of AHF once a month.

Episode 5 In June 1959 the patient was again admitted to hospital because of bleeding of the right knee. As long as the patient was receiving AHF once a month, he was in unusually good condition However in April and May 1959 the interval was increased to 6 weeks. Moreover the activity of the preparation used for the last injection in May was low and raised the patient's AHF to only 13 per cent. On admission *(June 1 1959)* the right knee had swollen to the *size of a football and was blue black. The* haematoma had developed in the course of 2—3 days. The patient had intense pain a body temperature *of 38.2 C and* a haemoglobin level *of 8.9 g/100* ml. He was treated with AHF and transfusion *of 500 ml of bank blood (Fig 13)*

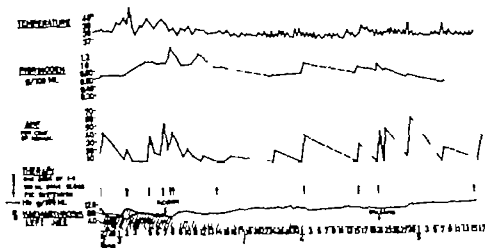


Fig. 12. Haemarthrosis (left knee) — episod 4 — in B.B., fam. 72, born 1946. Treatment and course

therapy in haemarthrosis, for the con dition is self-limiting, and it is not possible to know what th furthe course would have been if AHF had not been given. This is all the more difficult because treatment also included bed rest and it is obvious that early immobilization of the joints mu t have helped to limit haemorrhage It was rema kable however that all the patients who had received AHF therapy reported prompt relief of the pain in the respective joints. In additi n the swelling of the joints usually disappeared within the course of 2—4 days. No new joint deformitie de el ped in association with joint bleeding treated with AHF Several patients, who after previous haem rthrosis had developed joint deformities, have been treated with AHF in association with new bleed ing in the affected joints. The treat ment has gi en relief of pain The resolution of the haemarthrosis in these cases is difficult to evaluate and no improvement of the state of the joint could be observed. Some examples of the use of fraction I—0 in association with joint bleeding are given below

P \ *fam. 103 born 1945* — This is boy with severe haemophilia A, who had had several episodes of severe bleeding (see *Tooth extractions ,circumcisi for phimo is*) Since September 1958 he has been treated with AHF d he was given a total of 80 administrations between September 1958 and Jun 1961 (weight 35—47 kg) \ resistance t therapy has d veloped. This boy has been instructed to contact

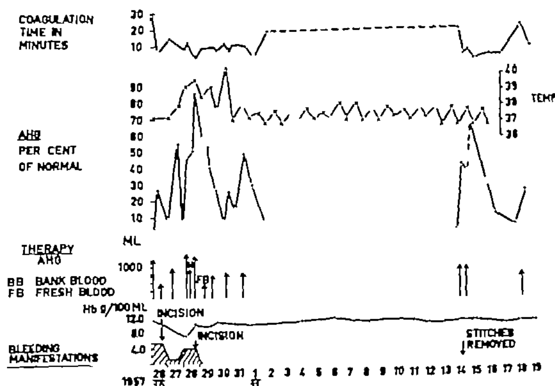


Fig. 11 Treatment and course in B.B., fam. 72, born 1946 (episode 3) (From Blombäck and Nilsson 1958)

fractured the left forearm with a dorsal deflection of 90 He was im mediately transported to his local hospital where he received a fresh blood transfusion and where the fracture was reduced He was then transferred to the hospital in Malmö On ad mission there 5 hours after the accident he was given half a dose of AHF daily for the next 4 days. No bleeding occurred and the fracture healed sa tisfactorily

R.F fam. 97 born 1926 — A 34 year old man with moderate haemophilia A had a motor car accident with fracture of the tibia and femur The fracture was reduced at his local hospital where he was also treated with 6 half doses of AHF Blood transfusions were also given No bleeding complications occurred. No further details have been reported to us.

Haemorrhage

Joint bleeding — Twenty-seven patients with haemophilia A (severe in 20 moderate in 6 and mild in 1) were treated with fraction I—0 because of haemarthrosis, mostly of the knee joints and the ankles 83 episodes of joint bleeding were treated (Table VI) with a total of 170 injections of AHF (88 doses) On each episode one to 8 half-doses of AHF were administered It is difficult to evaluate the effect of AHF

Table VI. Joint bleedings treated with fraction I—0

Joint	No. of episodes treated
Ankle	23
Knee	50
Hip	1
Shoulder	4
Elbow	5
Wrist	—
Total	83

6/61) The joint was markedly swollen, and he had severe pain. He received one t four injections of half-doses of AHF on each occasion The pain promptly disappeared. The AHF level increased after the i jection from less than 1 per cent to values between 15—40 per cent. The haemarthroses were resolved in the course of bout n week. The typical haemophili arthropathy has, however subsequently advanced in this left knee. H had on episod with joint bleeding in the left nkle nd right shoulder a d lbow (11/60) one episode with bleed ing in the left ankle (5/61) one episode w th bleed g in the left elbow (6/61) nd on with bleeding i the right ankle (7 61) On dmission the joints were ma kedly wollen and immobilized, a d he had severe pain. During each episode he received 2 to 4 i jection of half doses of AHF which rapidly resolved the haemarthrosis. H recovered full mob lity of these joints nd no permanent leformities have developed.

T.B., fam. 140 born 1956 — A 5 year old boy with severe haemophilia A. During the period 1958—1961 he had been admitted t hospital 29 times for bleed g from wou ds (3 times) epistaxis (2 times) haemarthrosis (5 times) ubcuta eous nd intramuscular bleedi g (7 times) and prophylactic treat ment with fraction I—0 (12 times) He has recei ed all together 17 administra-

Table VII Joint bleeding episodes treated with fraction I -0 in patient with severe haemophilia A (P V fam. 103 born 1945)

Date Month/ year	Joint bleeding	No. of administrations of I—0	No. of doses of I—0
10/58	Left knee	1	0.5
1/59	Left knee	1	0.5
4/59	Left knee	1	0.5
6/59	Left knee + left elbow	2	1
2/60	Right shoulder	2	1
3/60	Right knee	3	1.5
4/60	Left ankle	4	2
5/60	Left elbow	4	1.5
6/60	Left ankle	2	1
6/60	Right knee	4	2
7/60	Left knee	2	1
1/61	Left elbow	1	0.5
4/61	Left knee	3	1.5
5/61	Left knee	5	2.5
	Total	35	17

tions of fraction I—0 He has no impaired joint function.

Haematoma

Three patients with severe haemophilia A were treated because of large haematoma following injury

L.C fam. 64 born 1955 had a large inguin l haematoma after venipuncture of the femoral vein. After administration of 2 half-doses of AHF o more bleeding occurred. This case has been described (12 ase 4) *L.C.S., fam. 37 born 1958 and L.B fam. 105 born 1956* who both had severe haemophilia A, developed large haematomas after fall. The haematomas affected about half the thorax. They recei ed 4 and 2 half-doses of AHF respecti ely After dministration of AHF the haematomas did not in crease and they were resorbed within 4—5 days.

HAEMARTHROSIS (RIGHT KNEE) - EPISODE V
B B (Fam 72) 13 yrs (weight 48 kg)

FIBRINOGEN G/100 ML
TEMPERATURE C
AHF PER CENT OF NORMAL
THERAPY
ONE DOSE OF 1-0
500 ML BANK BLOOD
Hb g/100 ML
INCISION
HAEMARTHROSIS RIGHT KNEE
1/6 1959

Fig 13 Haemarthrosis (right knee) — episode 5 — in B.B fam 72, born 1946.

the hospital at the first sign of any joint bleeding, and on every occasion since October 1958 he has been given AHF It is clear from Table VII that he had had 14 episodes of joint bleeding. On each occasion he received ½ to 2½ doses of AHF The AHF level increased after half a dose of AHF from less than 1 per cent to 15—53 per cent. AHF gave prompt relief of pain the swelling decreased during the course of 2—7 days. He also recovered full range of mobility of the affected joints. This patient has not developed any haemophilic arthropathy and the range of movement of all joints is still normal

L.R C fam 74 born 1951 — This is a boy with severe haemophilia A, who since early childhood has been admitted to hospital repeatedly because of large haematomas, joint bleeding intra abdominal bleeding and bleeding after trauma. At 3 years of age he had severe bleeding from the left knee which resulted in a stretching defect Since then he has had repeated haemorrhages in this knee with consequent severe arthropathy with fixation in 90 flexion Since December 1950 the patient has been under our care (see *Reduction of bone fracture Intracranial haemorrhage Haematuria*) During this period he has had 8 episodes of severe joint haemorrhage *He had 4 episodes of bleeding in the left knee joint (3/60 5/60 8/60*

*) month/year

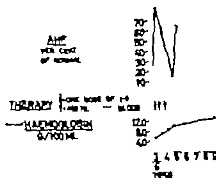


Fig. 14 Retroperitoneal haematoma in L.T fam. 75, born 1952. Treatment and course.

him. The next day he was better but the abdomen was still tender His temperature was 38.6 C. The following day he received half a dose of AHF The AHF level increased t 65 per cent. The haemoglobin value was 10 g/100 ml, he had abdominal pain and the abdomen felt normal. The fever disappeared He received no further therapy nd 4 d ys later he left the hospital in good co dition (Fig. 14)

R.K fam. 83 born 1945. — This was 16 year old boy with severe haemophil a A. H had had very severe bleeding symptoms since early childhood and had rece ved at least 100 blood transf sions. A irculati g anticoagulant had been dem nstrable since 1957 In July 1961 he was admitted to hospital with bleeding, hock, and symptoms of intra bdominal bleeding. He received immediately 2 whole doses of fraction I—0. The bleedi g did not stop. He died the following day Autopsy showed copiou retroperitoneal and i traabdominal bleeding.

Gastrointestinal haemorrhage

Thirteen patients, of whom 8 had severe 2 moderate and 3 mild haemophilia A were treated because of gastrointestinal bleeding (19 epi sodes) The patients with severe haemophilia A and one of those with moderate haemophilia A received 1 to 4 injections of AHF in combination with blood transfusion therapy (6 were treated at their local hospital) The effect of administration of AHF in these cases is difficult to evaluate, but the doctors reported a good haemostatic effect of fraction I—0 The patients with mild haemophilia A received 1 to 3 half doses of AHF which controlled the bleeding A patient with moderate haemophilia A, who has had several episodes of severe gastrointestinal bleeding, is reported in detail below

M.H fam. 27 born 1921 — First symptom of haemophilia t one year of ge (subcutaneous haemorrhages) Repeated haemarthroses in the nkle and knee joint during youth. In 1927 he had a large etroperitoneal haemorrhage and received repeated blood transfusions. In 1940 he had a bleeding in the bottom of the mouth. In 1947 he was treated in hospital for 2 months because of haematuria after a large haematoma in the right groin and received 20 blood transfusions. During the years 1949—1951 he was treated in hospital 3 times for haematoma nd haemarthroses after various traumata and received a total f 12 fresh blood transfusions. In 1951 he had his first gastrointestinal haemorrhage. He was treated in hospital for 6 weeks and received 58 fresh blood transfusions. Melaena persisted 2 weeks and then gradually subsided. X-ray examination showed no ulcer In November 1956 he had a new gastrointestinal haemorrhage He received 4 bottles of

Eleven patients with severe haemophilia A received AHF because of spontaneous haematoma (16 episodes) All these patients had large haematoma and severe pain on admission. During each episode they received one to four half doses of AHF Though it is difficult to say to what extent the AHF was responsible the haematomas never increased after administration of the preparation These patients obtained considerable relief of pain The haematoma rapidly disappeared No blood transfusions were necessary

Haemorrhage in the throat

Two patients with severe haemophilia A were treated with AHF in connection with haemorrhage in the floor of the mouth

R.A., fam 10 born 1953 — A 6 year old boy with severe haemophilia A (see *Major surgery*) He was admitted to hospital with a life-threatening haemorrhage in the floor of the mouth. He received 5 injections of AHF (3½ doses) during the course of 3 days. The bleeding stopped, and he recovered.

C.H.N fam 4 born 1957 — A 2 year old boy with severe haemophilia. He was treated at the hospital in Gothenburg and received half a dose of AHF because of laryngeal bleeding. This controlled the bleeding

Retroperitoneal haemorrhage

Ten patients were treated with AHF (12 episodes) for retroperitoneal haemorrhage Nine had severe haemophilia A and one moderate These patients were admitted to hospital because of severe abdominal pain Some of the patients were in a state of shock on admission The surgeons suspected appendicitis or peritonitis in all the cases. The patients had however also symptoms of bleeding with decreasing haemoglobin values. All of them were seriously ill On admission they had haemoglobin levels ranging between 3.5 and 8 g/100 ml The effect of administration of of AHF in these cases was obvious. The pain promptly disappeared and the patients showed no evidence of increasing haemorrhage On the contrary the abdomen soon felt normal and the haemoglobin level rose spontaneously A few illustrative cases are given below

U.T., fam 3 born 1957* — This patient a 4 year old girl" with male sex chromatin pattern has been reported earlier (55) (see also *Skin biopsy*) In May 1961 the patient was admitted to her local hospital with severe retroperitoneal bleeding The patient was treated at that hospital, from which the following report was obtained Admitted in poor condition with a haemoglobin value of 3.5 g/100 ml and RBC 1.3 mill. per cu mm. She received 400 ml of blood and 4 half-doses of AHF in the course of 2 days. The bleeding was completely controlled and she soon recovered.

L.T fam 75 born 1952. — A 6 year old boy with severe haemophilia A. He was admitted to the hospital on June 3 1959 because of 1 day's symptoms of appendicitis. He was pale and looked ill. The abdomen was tender and there was muscular defence as in peritonitis, particularly in the right iliac fossa. But there was also retroperitoneal tenderness, and a haematoma was beginning to develop in the right groin The haemoglobin value was 8.4 g/100 ml. His temperature was 38.3. He received one dose of AHF immediately The AHF level increased from less than 1 per cent to 83 per cent. He was also given a blood transfusion The surgeons suspected appendicitis and wanted to operate upon

1 June 1961 he again had a gastro-intestinal haemorrhage. The therapy is shown in Table VIII.

The patient had repeated melaena during the first 10 days, after which time it then ceased. He left hospital 3 weeks after admission.

Haematuria

Six patients were treated with AHF because of haematuria. Five of these patients had severe haemophilia A and one mild haemophilia A. After administration of a half or a whole dose of AHF the haematuria invariably ceased or became considerably less marked. G B *fam. 18 born 1912* has been described (18 case 2). Two illustrative case reports are given below.

N.G.W. fam. 18 born 1930 — A 31 yea old man who had had symptoms of severe haemophilia ever since early childhood. He was crippled as a result of joint bleedings. He had had at least 10 episodes of gross haematuria, which had usually lasted for weeks.

On June 1961 he had a new episode of gross haematuria. He received half a dose of AHF. The urine became clear within 6 hours.

L.H.C., fam. born 1951 (see *Joint bleeding M nor surgery I tracranial haemorrhage*). In June 1961 the patient was admitted t hospital because of gross haematuria. He received half dose of AHF. The haematuria ceased d no red blood cells were bserved in the uri (Fig. 16). The following d y he received half a dose of AHF. Two days later he again had gross h e maturia. After half dose of AHF th urin became clear. He received two further half-doses of AHF and was discharged from hospital. The haemoglobin value was 11.7 g/100 ml on admission and 12.4 g/100 ml when he was sent home.

Intracranial haemorrhage

Six patients with severe haemophilia A were treated with AHF in connection with intracranial haemorrhage. Four of the patients received only half a dose of AHF and the effect cannot be evaluated. One patient was treated in Poland, and he received 4 injections of AHF in con nection with head injury. A good haemostatic effect was reported. One case history is given below.

L.H.C., fam. 74 born 1951 — Episode I. An 8 year old boy with severe hae mophilia A (see *Minor surgery Joint bl eding Haematuria*). Weight 18 kg. On December 16, 1959 the boy bumped hi head against a radiator and felt ill for 6—8 hours afterwards. On admission to hospital in Malmö 2 days later the patient was drowsy he had stiffness of the neck, he was nauseated and had severe headache. The pupils were dilated and reacted only weakly t light, and he had right-sided abducens paresis. Roentgenologic examination of the skull showed no signs of a pathological condition. The condition was interpreted as i tracranial haemorrhage. He immediately eceived half a dose of AHF and then half a dose daily the following 6 d ys. Thereafter half a dose of AHF was given 3 times at 3 day intervals. During the first six days the AHF level ra ged between 15 and 33 per cent. The patient improved rapidly. The neck stiffness and headache disappeared after 2 days. The paresis and other neurological ympt ms also gradually abated. Within 3 weeks he was sent home symptom-free.

Fig. 15 Treatment and course in M.H., fam. 27 IV 8 born 1921 The amount of AHF (=AHG) injected is expressed as the volume of normal blood (ml) with corresponding AHF activity (From Blombäck and Nilsson 1958)

400 ml fresh blood with 65 ml ACD-solution per day on 5 consecutive days (total 20 bottles) but the bleeding persisted and the patient grew worse On the sixth day one whole dose of AHF preparation was given with an immediate effect (Fig 15) During the next 4 days the patient received 9 fresh blood transfusions, but the haemoglobin values did not rise On the 11th day after admission to hospital new profuse bleeding started and the patient fell into a state of shock He received one whole dose of AHF and his general condition rapidly improved. On the following 3 days he received a total of 5 fresh blood transfusions, and there was no further bleeding After 2 weeks he left the hospital with normal haemoglobin values.

In June 1958 he had a new gastrointestinal haemorrhage. During the first four days in hospital he was given 7 fresh blood transfusions, but the bleeding continued. On the fifth day he received one whole dose of AHF preparation and during the following five days two fresh blood transfusions a day The

Table VIII Treatment of gastrointestinal haemorrhage (episode IV) in patient M.H., fam. 27 born 1921

Date	AHF Doses 200 ml	Fresh whole blood Units 400 ml	Fresh plasma Units 400 ml	Concentrated erythrocyte suspension Units 200 ml
June 20	1	4	—	—
21	1+0.5 +1.5	6	—	—
22	1	4	—	—
23	1	3	—	—
24	1	2	1	—
25	1	3	—	2
26	1	5	—	—
27	1	3	—	—
28	0.5	2	—	—
29	—	—	1	—
30	1	—	—	—
July 1	—	—	1	—
2	—	1	—	—
3	—	—	1	—
4	—	1	—	—
Total	11.5	34	4	2

bleeding ceased and he left hospital after two weeks with normal haemoglobin values. He received a total of 90 blood transfusions.

The next gastrointestinal haemorrhage occurred in June 1960 The patient had bitten his tongue and bled for a week. He then had melaena and was admitted to hospital. The first three days he received a total of 5 fresh blood and 3 fresh plasma transfusions without effect. On the fourth day he received one dose of AHF and 5 fresh blood transfusions. On the fifth day he received half a dose on the ninth one whole dose on the fifteenth day one whole dose on the nineteenth day one dose of AHF From the fifth to the 25th day he received 49 fresh blood transfusions and one fresh plasma transfusion During the last 2 weeks in hospital he received 13 fresh blood transfusions.

In June 1961 he again had a gastro-intestinal haemorrhage. The therapy is shown in Table VIII.

The patient had repeated melaena during the first 10 days, after which time it then eased. He left hospital 3 weeks fter admission.

Haematuria

Six patients were treated with AHF because of haematuria. Five of these patients had severe haemophilia A and one mild haemophilia A. After administration of a half or a whole dose of AHF the haematuria invari ably ceased or became considerably less marked. *G B fam 18 born 1912* has been described (18 case 3) Two illu trative case reports are given below

\.G W fam. 18 born 1930 — A 31 year old man who had had symptoms of severe haemophilia ever since early childhood. He was crippled as a result of joi t bleedings. He had had at least 10 episodes of gross haematuria, which had usually lasted for weeks.

On June 1961 he had a new episod of gross haematuria. He received half dose f AHF The urine became clear withi 8 hours.

L.R.C fam. born 1951 (see *Joint bl eding Minor surgery Intracranial haemorrhage*) In June 1961 the patient was admitted t hospital because of gross haematuria. H received half a dose of AHF The haematuria ceased, and no red blood cells were observed in the urine (Fig. 16) The following day he received half a dose of AHF Two d y later he again had gross haematuria. After half a dose of AHF the urine became clear He received two furthe half-doses of AHF and was discharged from hospital. The haemoglobin value was 11 7 g/100 ml on admission and 12.4 g/100 ml when he was sent home.

Intracranial haemorrhage

Six patients with severe haemophilia A were treated with AHF in connection with intracranial haemorrhage Four of the patients received only half a dose of AHF and the effect cannot be evaluated One pa tient was treated in Poland and he received 4 injections of AHF in connection with head injury A good haemostatic effect was reported. One case history is given below

L.R.C fam. 14 born 1951 — Episode I An 8 year old boy with severe haemophilia A (see *Minor surgery Joint bleeding Haematuria*) Weight 18 kg. On December 16, 1959 the boy bumped his head against a radiator and felt ill f r 6—8 hours afterwards. On admission to hospital in Malmö 2 day later the patient was drowsy he had stiffness of the neck, he was nauseated and had severe headache The pupils were dilated a d reacted nly weakly to light, and he had right-sided abducens paresis. Roentgenologic examination of the skull showed no signs of a pathological condition. Th conditi n was interpreted as intracranial haemorrhage. H immediately received half a dose of AHF and then half a dose daily the following 6 days. Thereafte half a dose of AHF was given 2 times at 3 d y intervals. During the first six days the AHF level ranged between 15 and 32 per cent. The patient improved rapidly The neck stiffness and headache disappeared after 2 days. The paresis and other neurological symptoms also gradually abated. Within 3 weeks he was sent home symptom-free.

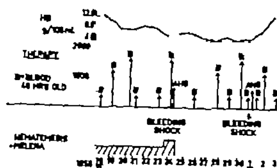


Fig. 15 Treatment and course in M.H., fam. 27 IV 8 born 1921 The amount of AHF (=AHG) injected is expressed as the volume of normal blood (ml) with corresponding AHF activity (From Blombäck and Nilsson, 1958)

400 ml fresh blood with 65 ml ACD-solution per day on 5 consecutive days (total 20 bottles) but the bleeding persisted and the patient grew worse. On the sixth day one whole dose of AHF preparation was given with an immediate effect (Fig. 15) During the next 4 days the patient received 9 fresh blood transfusions, but the haemoglobin values did not rise. On the 11th day after admission to hospital new profuse bleeding started and the patient fell into a state of shock. He received one whole dose of AHF and his general condition rapidly improved On the following 3 days he received a total of 5 fresh blood transfusions, and there was no further bleeding. After 2 weeks he left the hospital with normal haemoglobin values.

In June 1958 he had a new gastrointestinal haemorrhage During the first four days in hospital he was given 7 fresh blood transfusions, but the bleeding continued On the fifth day he received one whole dose of AHF preparation and during the following five days two fresh blood transfusions a day The

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22	1	4	—	—
23	1	3	—	—
24	1	2	1	—
25	1	3	—	2
26	1	5	—	—
27	1	3	—	—
28	0.5	2	—	—
29	—	—	1	—
30	1	—	—	—
July 1	—	—	1	—
2	—	1	—	—
3	—	—	1	—
4	—	1	—	—
Total	11.5	34	4	2

bleeding ceased and he left hospital after two weeks with normal haemoglobin values. He received a total of 20 blood transfusions.

The next gastrointestinal haemorrhage occurred in June 1960 The patient had bitten his tongue and bled for a week. He then had melaena and was admitted to hospital. The first three days he received a total of 5 fresh blood and 3 fresh plasma transfusions without effect. On the fourth day he received one dose of AHF and 5 fresh blood transfusions. On the fifth day he received half a dose on the ninth one whole dose on the fifteenth day one whole dose on the nineteenth day one dose of AHF From the fifth to the 25th day he received 49 fresh blood transfusions and one fresh plasma transfusion. During the last 2 weeks in hospital he received 13 fresh blood transfusions.

I.P fam. 127 born 1881. — A 79 year old man with mild haemophilia A. During a hernia operation in 1937 h bled nearly to death, and after tooth extraction he had always bled for a long time and had t be admitted to hospital.

In June 1958 he was dmitted to his local hospital because of calculus in the urinary bladder Attempts were made t remove the calculus by crushing it under cystoscopy but unfortunat ly the wall of the rectum was perforated and a urinary rectal fistula developed The patient was admitted to hospital in Stockholm for operation. It was not possible to close the fistula and the p tient bled profusely and received blood transfusions.

In September 1958 he was gain admitted to hospital in Stockholm for new ttempt t close the fistula. This operation was successful and there was early postoperative haemorrhage. On the day after operation there was slight bleeding through the urinary drain, which ceased pontaneously On the fifth day after the operation there was profuse bleedi g from th drain. Coagulation tests showed a low AHF acti ity (3.7 per cent) and therapy with fraction I—0 was started the same day he received one whole dose of AHF H was reoperated upon and no local se of the bleeding was found. The wound was losed d one whole dose of fraction I—0 was given. On the third day after the second operation he received on whole dose and on the fifth, seventh and twelfth day half a dose of AHF H was also given 22 blood transfusion d 8 plasma transfusions during this 2-week period. From the third to t th day after the second operation the AHF value was about 15—25 % of normal. There wa no bleeding from the wound.

Recovery was slow and in October 1958, one month after the second operation, while still in hospital, th patient had profuse haematemesis and melaena and went into shock. He was given half a dose of AHF and blood transfusions, and th bleeding stopped. He recovered lowly but completely

P V fam. 102 born 1945 — This patient with severe haemophilia A has been described above (see *Tooth extractions Joint bleeding Operation for circumcision*) In March 1961 he was admitted to hospital because of profuse bleeding from a cut wound of the forearm. He received 3 half-doses of AHF Th AHF level was kept between 17 and 33 per cent. The bleeding stopped promptly and th wound healed without complications.

L.B fam. 72 born 1948. — A 12 year ld boy with severe haemophilia A (see *Prophyla ti treatment*) About half a day aft r a venipuncture he started to bleed profusely from the site of the procedure H was admitted to hospital in a state of shock and with a haemoglobin value of 9 g/100 ml. He was immediately given ne whole dose of AHF The bleedi g stopped promptly The following day he recei ed half a dose of AHF N more bleeding occurred.

Haemorrhage after puncture of haemarthrosis

T.S. fam. 32 born 1953. — This patient with severe haemophilia A has been described above (see *Tooth extraction Skin biopsy*) Weight 23 kg On August 25 1960 the patient was admitted t his local hospital because of a severe haemarthrosis f the left knee. He recei ed a transfusion of fresh blood, fter which the joint was punctured. The knee was straightened out under anaesthesia and immobilized. This caused severe bleeding into the knee joint. The haemarthrosis was more marked than before puncture. The patient received another blood transfusion.

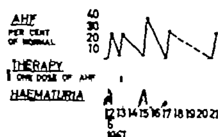


Fig. 16 Gross haematuria in L.R C., fam. 74 born 1951 Treatment and course.

Episode II Four months later the patient was again admitted to hospital because of a head injury he had sustained when falling down stone steps. He was drowsy he had neck stiffness and vomited He had severe headache, and probably choked discs. On the day of admission he received 2 half-doses of AHF and then half a dose on 4 consecutive days, and then a further 4 half-doses at 3 day intervals. During the first few days the AHF level was kept between 10—53 per cent. He soon became better The headache the neck stiffness and the papillary congestion disappeared and no further neurological symptoms developed

Haemarrhage after tooth extractions

Two patients with moderate haemophilia A were admitted to the hospital because of life-threatening bleeding after tooth extraction which had been performed without adequate measures against haemorrhage

E.N., fam. 111 born 1929 — In this patient 5 teeth had been extracted at his local hospital He bled diffusely and went into shock. He received blood transfusions and had a transfusion reaction with uremia It was later shown that his serum contained anti-c in a high titer He received 6 injections of AHF (4 doses) and the bleeding was controlled (Fig. 17) The patient recovered completely This case has been reported in detail (18 case 7)

R.F., fam 87 born 1939 — A 10 year old boy with moderate haemophilia A (see *Major surgery Tooth extractions*)

In February 1959 he had a loose tooth in the upper jaw As the tooth was movable the dentist thought that the extraction could be performed without complications. Immediate haemostasis was good but the following day blood began to ooze from the socket An upper respiratory tract infection developed with fever between 38—39 C. The following days he bled intermittently and on the seventh postoperative day he was in very bad condition and was admitted to hospital He then had a haemoglobin level of 3.5 g/100 ml.

He was immediately given 200 ml of fresh blood and half a dose of fraction I—0 The bleeding promptly stopped, and he received a further 200 ml of fresh blood that day The next day the plasma AHF activity was 8 per cent, and he was given half a dose of AHF The extraction wound healed rapidly and he left the hospital after a week with a haemoglobin level of 11.2 g/100 ml.

Haemorrhage after surgery and cut wounds

One patient with mild haemophilia A was treated for profuse bleeding following surgery and two patients with severe haemophilia A due to profuse bleeding after cut wounds

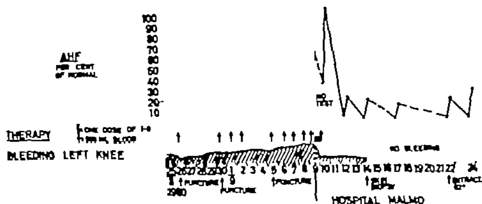


Fig. 18. Bleeding after puncture of joint bleeding in T.S., fam. 32, born 1953. Treatment and course

Nose bleeding

Three patients with severe haemophilia A and one with mild haemophilia A were treated due to prolonged severe nose bleeding

C.S., fam. 37 born 1951 and L.C.S fam. 37 born 1955. — Two brothers with severe haemophilia A. They were admitted to hospital because of prolonged profuse nose bleeding They received 2 half-doses and 1 half-dose of AHF respectively The bleeding stopped promptly

R.K fam. 88 born 1915. — Severe haemophilia A with circulating anticoagulant (see *Retroperitoneal bleeding*) He was admitted to hospital with profuse nose bleeding and back. After administration of 7 half doses of AHF the bleeding was controlled.

Prophylactic treatment

Two brothers, B.B., born 1946 and L.B born 1947 fam. 72 with extremely severe haemophilia A (see *Joint operations Bleeding after cut injury*) have received half dose of fraction I—0 prophylactically once a month since May 1958, i.e a total of 3½ years. These boys had previously been admitted to hospital several times every year with severe bleeding requiring several blood transfusions or since 1956, treatment with fraction I—0 Tables IX and X summarize the days of hospitalization each year and the therapy given. During the period of prophylactic treatment they were in much better condition than before and were able to do school work. During this period the older boy had episodes of bleeding in the knee joint (see *episode 5 Joint operation*) As reported earlier this boy had previously been treated with AHF in connection with 4 episodes of severe bleeding with necrosis in the joints. These latter bleeding episodes were much less serious. In addition this boy had one episode of retroperitoneal bleeding, from

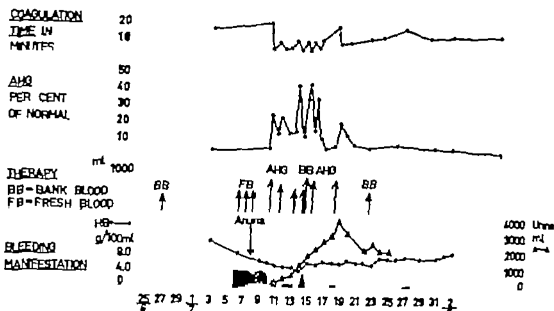


Fig. 17 Treatment and course in E.N., fam. 111 born 1929 The amonnt of AHF (=AHG) injected is expressed as the volume of normal blood (ml) with corresponding AHF activity (From Blombäck and Nilsson 1958)

and the knee was re-punctured (August 30) Puncture had no effect on the haemarthrosis the joint swelled subsequently In addition blood oozed continuously from the needle track He was given 3 blood transfusions. On September 5 the knee was punctured again This resulted in considerable bleeding into the joint and haemorrhage from the needle track. The patient received 3 blood transfusions, but the bleeding did not stop. On September 9 he received a blood transfusion and was transferred to the hospital in Malmö (Fig. 18)

On admission in Malmö (September 9) the patient was in poor general condition and the *left knee was bleeding. Half a dose of AHF was given immediately* The AHF level increased to 65 *per cent and the bleeding stopped* promptly (Fig. 18) He was given a further half a dose of AHF and a transfusion of fresh blood. The next day the AHF level was 30 per cent. He was given half a dose of AHF and the AHF level increased to 105 per cent His general condition was much better During the later course he was given a further 2 doses of AHF (Fig. 18) No further bleeding occurred and the swelling of the left knee gradually disappeared The patient received massage and physical exercise under cover of AHF and recovery of the mobility of the joint was satisfactory The range of motion of this joint is now normal. While in hospital skin biopsy was done and a tooth was extracted without bleeding complications.

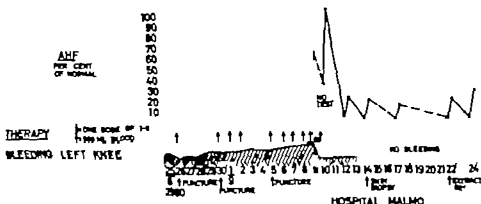


Fig. 18. Bleeding after puncture of joint bleeding in T.S., fam. 32, born 1953. Treatment and course.

Nose bleeding

Three patients with severe haemophilia A and one with mild haemophilia A were treated due to prolonged severe nose bleeding.

C.S., fam. 37 born 1951 and L.C.S., fam. 37 born 1958. — Two brothers with severe haemophilia A. They w re admitted t hosp tal because of prolonged profuse nose bleeding. They received 2 half-doses and 1 half-dose of AHF respectively The bleeding stopped promptly

R.K. fam. 88 born 1945 — Severe haemoph lia A with ci culating anticoagula t (see *Retroperitoneal bleeding*) H was admitt d t hospital with profuse nose bleeding and shock. After admi istrat on f 7 half doses of AHF the bleed ng was ontrolled.

Prophyl ctic tre tment

Two broth rs, B.B., born 1946 and L.B., born 1947 fam. 72 with extremely severe haemophilia A (see *Joint operations Bleeding after cut injury*) ha e received half dose of fraction I—0 prophylactically once a month since May 19 8, *i.e* a total of 3½ years. These boys had previously been admitted to hospital several times every year with severe bleeding requiring several blood transfusions or ince 1956, treatment with fraction I—0. Tables IX and X summarize the days of hospitalization each year and the therapy given. During the period of prophylactic treatment they were i much better condition than before and were able to d school work. During this period the lder boy had 2 episodes of bleeding in the knee joint (see episod 5 *Joint operation*) As reported earlier this boy had previously been treated with AHF in connection with 4 episodes of severe bleeding with necrosis in the joints. These latter bleeding episodes were much less serious. In addition this boy had one episod of retroperitoneal bleeding, from

Table IX. Prophylactic treatment with fraction I—0 of patient B.B., fam. 72 born 1946

Year	Age yrs	Days of hospitalization	Prophylactic treatment (half a dose of fraction I—0 per month)	No. of blood trans.	No. of doses of fraction I—0 for Bleeding episodes	Prophylactic treatment
1946	/	8	—	—	—	—
1947	1	11	—	1	—	—
1948	2	12	—	1	—	—
1949	3	3	—	1	—	—
1950	4	28	—	2	—	—
1951	5	34	—	5	—	—
1952	6	0	—	0	—	—
1953	7	50	—	3	—	—
1954	8	70	—	5	—	—
1955	9	279	—	23	—	—
1956	10	69	—	10	8.5	—
1957	11	26	—	0	8	—
1958 Jan.—April	12	91	—	7	13	—
1958 May—Dec.	12	0	May—Dec.	0	0	3
1959	13	56	Jan.—April and July—Dec.	8	12	4.5
1960	14	0	Jan.—Dec.	0	0	6.5
1961	15	32	Jan.—Oct.	2	14.5	4.5

Table X. Prophylactic treatment with fraction I—0 of patient L.B., fam. 7 born 1948

Year	Age yrs	Days of hospitalization	Prophylactic treatment (half dose of fraction I—0 per month)	No. of blood transf.	No. of doses of fraction I—0 for Bleeding episodes	Prophylactic treatment
1950	2	24	—	2	—	—
1951	3	13	—	3	—	—
1952	4	19	—	0	—	—
1953	5	16	—	0	—	—
1954	6	13	—	0	—	—
1955	7	12	—	2	—	—
1956	8	14	—	4	—	—
1957	9	30	—	2	2	—
1958	10	0	Oct.—Dec.	0	0	1.5
1959	11	14	Jan.—April and June—Dec.	2	0.5	5.5
1960	12	4	Jan.—Dec.	2	3	6.5
1961	13	0	Jan.—Oct.	0	0	5.5

which he recovered within 11 days. Otherwise he had no severe bleeding and the haemophilia appeared much less se-ere than before. One f the episodes of joint bleeding developed when the inter val between th injections was increased to 6 weeks the retroperitoneal bleeding developed one week after the patient had received a less active dose. This older boy has received the fraction 107 times in the course of 5 years, but no resistance to therapy has developed. After administration of fraction I—0 the AHF level increased to values ranging between 15 and 50 per cent. It is true that the AHF level decreased rapidly but the residual AHF fell very slowly so that the patient's AHF level was 1—3 % instead of 0.1 % for almost month.

The younger boy has received prophy lactic treatme t since the age of 10 years (since May 1958) During this period he has spent only 18 days in hospital once because of a haematoma in the thigh and once because of bleeding after veni puncture. He has had episodes of bleed ing i the knee joints, ankles nd elbows. These joint bleedings have not been severe, nd no changes of the type in the older boy have as yet developed. This boy has slight deformity of the left knee but the range of motion is normal. S nce 1957 he has received the fraction 40 times. No resistance to therapy has developed. The AHF level before treatment is in this boy also in the range f 1—2 %.

T.B., fam. 140 born 1954. — This boy with severe haemophilia A has received prophylacti treatment, half dose of AHF once month since May 1959 (see *Joint bleeding*) During this period h has had several joint bleedings and haematomas f llowing various traumata. However the joi t bledings have been quickly bsorbed nd no deformities have dev loped. He has received fraction I—0 prophylactically 12 times (6 doses)

Discussion

Since 1956 fraction I—0 has now been administered 818 times (497 doses) to 63 patients (59 Swedish patients) Of the Swedish patients 40 had severe 12 moderate and 7 mild haemophilia A. One dose of fraction I—0 is prepared from 1400 to 1600 ml of fresh normal plasma. Usually half a dose or one whole dose of fraction I—0 was given on each occasion (Table II) Forty-six of the patients received repeated administrations of fraction I—0 Thus 11 patients received 20 or more infusions of I—0 One patient received 107 infusions of fraction I—0 between June 1956 and June 1961

No untoward reactions to the infusions have been observed No fever and no anaphylactic reaction have been recorded, not even in those patients who received more than 100 infusions. No signs of haemolysis or other changes in the blood picture have been noted. Two patients developed hepatitis *(K S fam 45 born 1904* and *L. C R fam. 74 born 1951)* These patients had received 118 and 55 infusions of fraction I—0 respectively The first patient also received 105 blood transfusions during the eight months before the onset of hepatitis. Both patients recovered from the hepatitis within 2—3 months.

It thus appears that fraction I—0 has produced fewer side effects than other AHF concentrates used. The animal AHF preparation of Bidwell (7 8) used by Macfarlane and Biggs and their co-workers are antigenic (51 35 48, 50 10 83) After administration f animal AHF severe anaphylactic reactions have been observed.

Table IX. Prophylactic treatment with fraction I—0 of patient B.B fam. 72 born 1946

Year	Age yrs	Days of hospitalization	Prophylactic treatment (half dose of fraction I—0 per month)	No. of blood-trans.	No. of doses of fraction I—0 for Bleeding episodes	Prophylactic treatment
1946	1/	8	—	—	—	—
1947	1	11	—	1	—	—
1948	2	12	—	1	—	—
1949	3	3	—	1	—	—
1950	4	28	—	3	—	—
1951	5	34	—	5	—	—
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1955	9	279	—	23	—	—
1956	10	69	—	10	6.5	—
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1958 Jan.—April	12	91	—	7	13	—
1958 May—Dec.	12	0	May—Dec.	0	0	2
1959	13	56	Jan.—April and July—Dec.	8	13	4.5
1960	14	0	Jan.—Dec.	0	0	6.5
1961	15	32	Jan.—Oct.	2	14.5	4.5

Table X. Prophylactic treatment with fraction I—0 of patient L.B fam 72 born 1948

Year	Age yrs	Days of hospitalization	Prophylactic treatment (half dose of fraction I—0 per month)	No. of blood-transf.	No. of doses of fraction I—0 for Bleeding episodes	Prophylactic treatment
1950	2	24	—	2	—	—
1951	3	13	—	3	—	—
1952	4	19	—	0	—	—
1953	5	16	—	0	—	—
1954	6	13	—	0	—	—
1955	7	12	—	2	—	—
1956	8	14	—	4	—	—
1957	9	30	—	2	2	—
1958	10	0	Oct.—Dec.	0	0	1.5
1959	11	14	Jan.—April and June—Dec.	2	0.5	5.5
1960	12	4	Jan.—Dec.	2	2	6.5
1961	13	0	Jan.—Oct.	0	0	5.5

to prepare fraction I—0 containing AHF in an amount sufficient to increase the plasma AHF from 0 to about 30 per cent of normal in a person weighing 70 kg Furthermore, after administration of fraction I—0 the coagulation time became normal The prothrombin consumption test also became normal. The fibrinogen level increased Infusion of one dose of fraction I—0 increased the fibrinogen level in an adult 0.05—0 1 g/100 ml In some patients who received repeated doses of AHF at close intervals the fibrinogen level increased to levels as high as 1 4 g/100 ml (see Figs. 2, 3, 12 and 13) In most of our patients the fibrinogen levels did not show such significant increases as in the cases reported by McMillan et al. (52) It might depend upon the fact that to achieve a certain level of AHF they had to administer a larger amount of protein No undesired effects of the high fibrinogen levels were observed in our patients. Never did the high fibrinogen level prevent continuation of the desired treatment The platelet count did not decrease. No change occurred in the fibrinolytic activity as measured on unheated fibrin plates (66) Neither were changes observed in other coagulation factors.

The AHF activity in fraction I—0 per mg of protein is 20 to 50 times that of plasma (12, 16 15) The bovine AHF fraction prepared by Bidwell (7 8) is 100—400 times as active as the original plasma per mg nitrogen The human preparations of Kekwick and Wolf (39) have an AHF activity of 20—25 times that of the original plasma per mg protein. McMillan et al. (52) have not given the purification factor for their AHF fraction. From their figures the activity as judged from *in vivo* tests, may be 10—20 times that of plasma per mg protein

Clinical Effect

A review of the 63 cases in which human fraction I—0 has been used allows assessment of the therapeutic value of the preparation The patients were treated in connection with gastrointestinal, retroperitoneal, renal and joint bleeding, haematomas and bleeding following surgery and injury and minor surgical procedures such as aspiration of a haemarthrosis, tooth extraction, incision of phlegmon reduction of bone fracture and operation for phimosis. Major surgery including appendectomy operation for ileus, ureterolithiasis and nephrolithiasis, nephrectomy and cholecystectomy was performed under cover of fraction I—0 8 times in 6 patients, of whom 3 had severe and 3 moderate haemophilia. Administration of fraction I—0 arrested abnormal bleeding and surgical procedures could be performed without any haemorrhagic complications. It was quite obvious from our study that the AHF level of the plasma of the patient had to be raised to a certain haemostatic level in order to control bleeding episodes. In connection with surgical procedures we found it suitable to increase the plasma AHF to 40—80 per cent of normal before operation and then to maintain it at about 30—40 per cent during the first two postoperative days and at 20—30 per cent during the healing period. In order to control a severe bleeding episode such a gastrointestinal, retroperitoneal and posto-

Macfarlane and co-workers have also reported fever and shivering in connection with infusion of animal AHF as well as a decreased number of platelets in some cases and urticaria in other cases after repeated administrations of heterologous AHF Kekwick and Wolf (39) and Wolf (95 96) using a fraction prepared from human plasma by the ether water fractionation process of Kekwick Mackay and Record (38) have reported transfusion reactions in about 9 % of their cases The most common reactions were flushing of the face headache conjunctival irritation pain in the back or abdomen and a feeling of constriction of the chest irregular pulse and occasionally urticaria and vomiting We did not see any such reactions McMillan Diamond and Surgenor (52) who used a fraction similar to that used in this study reported apneic episodes in one child of their 15 cases In 2 patients haemolytic phenomena concomitant with high fibrinogen levels were observed Hepatitis developed in one patient Otherwise no side effects were noted

The most common drawback of the treatment of haemophiliacs with plasma fractions is a refractory period when the response to repeated injections is absent or less than expected No diminished clinical response to therapy has been observed in our patients not even in those who have received the largest number of infusions (Table IV) No circulating anticoagulant could be demonstrated No diminution in the postinfusion rise of plasma AHF was observed after repeated series of infusions The effect of each preparation was compared with that of a dose from the same batch on the AHF level in an other patient Macfarlane and Biggs have reported resistance to treatment with animal AHF (51 50 10) Wolf (95 96) did not observe any resistance in 25 haemophiliacs treated repeatedly with the ether fractionation preparation McMillan et al. (52) reported development of resistance to replacement therapy after administration of fraction I in a single case in a patient with severe hepatitis and liver failure.

Forty seven of the haemophiliacs given fraction I—0 were treated in Malmö and Stockholm In these patients coagulation analyses were with few exceptions performed before and after injection of AHF In connection with the first 200 administrations the following analyses were performed coagulation time bleeding time platelet count, prothrombin consumption test prothrombin and factor VII factor V B factor AHF fibrinogen and fibrinolytic activity In connection with the later infusions we determined the coagulation time the AHF level and usually the fibrinogen level. As mentioned the plasma AHF activity of the patients was followed by the recalcification method on haemophilia A plasma and expressed as a percentage of that of plasma from a control person whose AHF level was the same as the mean of that of 20 healthy persons.

After administration of fraction I—0 the plasma AHF level increased, usually to the same or somewhat higher levels than those predicted by *in vitro* studies The mean *in vivo* yield of AHF in the best series was about 100 per cent of normal human plasma (13 16) Thus, when starting from 1 600 ml of fresh human plasma it was possible

vivo response was considerably lower than the expected value

Stefanini, Broderick, Gobbi and White (86) performed a complex orthopaedic operation on a child with severe haemophilia A The patient received 10 pints of fresh blood, 500 ml of packed red cells, 15,000 ml of fresh, frozen plasma, 4 units of concentrated AHF and bovine cephalin The operation was ultimately successful but severe bleeding complications occurred and they pointed out that surgery should be undertaken only in mild cases or under exceptional circumstances. McMillan et al. (52) performed 4 operations under cover of their fraction I. Two of these cases were complicated by bleeding

It is clear from our case reports that *major surgery* is possible even in severe cases of haemophilia A. All our patients recovered Of the 8 operations performed the course was uneventful in 5 (appendectomy cholecystectomy exploratory laparotomy operation for ileus and ureterolithot my) The AHF level was kept above 30 per cent during operation and the first postoperative days, and then between 15—30 per cent until the skin utures were removed. This was achi eved in an adult by giving one and a half doses or two doses of fraction I —0 just before operation, one or half dose 3 to 10 hours after the opera tion one or two doses on each of the next day and then half a dose 3 to 8 times during the postoperative course. We feel it is of utmost importance to continue the therapy for at least 10 t 14 days after the operation. This has also been pointed out by Caxal Izarn and Paleirac (22)

One of the operations for ureterolithiasis *(G P., fam 136 born 1908)* and the operation for ileus *(G B., fam 18 born 1912)* were performed at other hospitals where the AHF level was not followed.

The patient *K S fam 45 born 1904* in whom 3 operations includ ing nephrolithotomy nephrectomy and ureterolithotomy were performed, illustrates the difficulties encount ered in operation on haemophiliacs. The first operation, nephrolithotomy was performed without complicating haemorrhages. The AHF level was kept at 50—70 % during operation and the first postoperative day AHF levels between 15—40 % were then achieved for a further 8 days. Nephrectomy had to be performed later owing to refractory haematuria. As was apparent from the case report, it was found that he had an aneurysm in the kidney During this major operation the AHF content was kept between 50 and 80 % and the operation was not complicated by haemorrhage. The following afternoon the AHF level was 20 % The next day he had symptoms of intraabdominal bleeding in spite of the fact that the AHF level then was about 50 %. It is possible that this bleeding had started the day before. The AHF was rapidly consumed and it was difficult to keep at a ufficiently high level. When the AHF decreased to 10—15 % he had oozing bleeding from the wound, which stopped after administration of I—0 When he was operated upon (ureterolithotomy on the remaining kidney) one year later he had uraemia. No bleeding occurred in connection with this operation.

Although it is possible to operate upon patients with haemophilia A

perative bleeding in a patient with severe haemophilia we have found it advisable to increase the plasma AHF level to 40—60 per cent of normal immediately and then to maintain it at about 15—30 per cent until healing is complete. In the management of spontaneous haemorrhagic episodes such as joint bleeding and haematoma an AHF level of 10—20 per cent seems to be sufficient These figures are in agreement with those found by Biggs and Macfarlane (11)

The problem of surgery in haemophilia is well known (65) In 1948 Craddock Fenninger and Simmons (25) compiled the statistics of published cases and reported a 27 per cent mortality when surgery was performed on haemophiliacs. Successful operations on haemophiliacs have been reported but in most of the cases the postoperative course has been extremely stormy (21 28 82 94 90 89) In 1950 Pieper Perry and Burroughs (71) analysed the experiences of various authors with the problem of major surgery on haemophiliacs. Of 50 persons suspected of having haemophilia and in whom major surgical procedures were performed the mortality was 20 per cent In 24 such cases with proved haemophilia there was a similar mortality A mortality of 28 6 per cent was found for appendectomy in proved haemophiliacs These authors described two haemophilic patients subjected to appendectomy and goniotomy Infusions of fresh plasma collected by the silicone technique were given The goniotomy was performed on a 7 month old boy without bleeding while the appendectomy in an adult was followed by haemorrhage but both patients recovered

As already mentioned Macfarlane and Biggs (51 50 10) have used animal AHF in 14 patients submitted to a total of 17 operations In most of these operations bleeding was not excessive. They point out that the minimum safe level of AHF for surgery is 25 per cent. Biggs (10) says that the availability of animal AHF has made surgery on haemophiliacs a reasonably safe undertaking but one which should nevertheless not be undertaken lightly There is furthermore doubt as to the possibility of a second course of treatment and therefore patients may have only one chance of undergoing a major operation. Handley Painter and Hall (36) successfully used animal AHF to control bleeding in two haemophilic patients who underwent gastrojejunostomy and selective vagotomy for duodenal ulcer In both patients porcine AHF had to be discontinued after the 9th day One patient had an anaphylactic shock the other became refractory even to large doses of AHF The treatment period could be prolonged to about 12—14 days by using AHF from a different type of animal After this, further cover could be secured only by using fresh blood plasma, or human AHF concentrate Christie Graham Stewart and Ingram (23) reported a good result with animal AHF in connection with an operation on a haemophiliac. Egeberg Borchgrevink and Hjort (32) tried bovine and porcine AHF in a case of severe haemophilia A in connection with exarticulation in the hip The patient survived the operation but he bled seriously for several weeks. They pointed out that the turnover rate of the animal AHF did not differ from that of human AHF However the in

vivo response was considerably lower than the expected value

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under cover of fraction I—0 we think it advisable to operate only when absolutely necessary especially if the haemophilia is severe If however *operation* is unavoidable the patient should be carefully investigated beforehand even if such examinations require AHF in order to prevent as far as possible unexpected complications at operation A large supply of fraction I—0 must be available In the case reported above *(K S., fam 45 born 1904)* renal angiography under *cover of* fraction I—0 could have demonstrated *the renal aneurysm*

In 2 cases the patients were operated upon because of assumed appendicitis which later proved to be retroperitoneal haemorrhages. We think it advisable in such cases first to treat the patients with AHF and then to give expectant treatment only Not unless the abdominal symptoms increase in intensity without signs of bleeding should operation be considered

From our case repors it is apparent that *minor surgical* procedures can be performed without bleeding complications, provided the *AHF level is kept* at about 20 per cent of normal for a sufficient period We feel it is of importance to have an effective regimen for performance of minor surgical procedures in haemophiliacs. Extraction of teeth in a patient with haemophilia is an operation which most dental surgeons shun It is almost always followed by undue bleeding, which may persist for weeks. The amount *of* blood lost at the operation is usually not abnormal but within a few *hours bleeding* recurs and it may persist so that large quantities of *blood may be lost E N., fam 111 born 1939 and R F fam 87 born 1951* in whom teeth were extracted without precautions, show that tooth extraction can lead to serious bleeding problems Wishart Smith Honey and Taylor (93) reported in 1957 a technique for the removal of teeth from haemophiliacs This involved the use of transfusions of fresh plasma and well fitting acrylic dental splints They found that the bleeding after dental extractions can then be reduced or occasionally prevented. *Similar techniques have been used by* Rubin Levine and Rosenthal (81) *and Findlay and Nicholl (33)* Macfarlane and Biggs (60) have treated many patients for dental extraction with transfusions of fresh frozen plasma They gave 1000 ml of plasma as a routine measure to adults before the extraction This treatment raised the AHF level to 10—25 per cent and it was repeated as often as thought necessary According to their experience such treatment very seldom reduces bleeding to the normal level and troublesome bleedings were *not* uncommon They performed dental extractions successfully under cover *of animal AHF Kekwick and Wolf* (39) have performed dental extractions under cover of *their ether fractionation* preparation They reported some oozing bleeding but otherwise no bleeding complication Similar results were described by McMillan et al (52)

We have performed dental extractions in 5 patients with severe haemophilia A In patient *P I., fam 103 born 1915* a molar was first extracted without any bleeding complication. The AHF level was kept between 20 and 60 per cent of normal for 5 days He received 7 injections of *AHF* (4

whole doses) One month later two molars were extracted, one 2 days after the other (Fig. 6) The AHF level was between 10 and 40 per cent which was apparently not high enough, for bleeding occurred. This was promptly controlled by further doses of fraction I—0 The AHF level was kept between 20 and 70 % from the seventh to the tenth day after the extractions. There was no bleeding, but on the seventh day when the AHF level dropped to 10 % bleeding recurred. In connection with these extractions he received 23 injections of AHF (14 1/2 doses) In this case it was apparent that satisfactory haemostasis required an AHF level above 20 % for at least 10 days after extraction of two molars. In this case the AHF also appeared to be consumed more rapidly in connection with the bleeding. It also seemed that the emotional state of the patient enhanced the bleeding tendency In connection with blood transfusions he had chills, and then much lower AHF levels were noted. The patient *L T f m 75 born 1952* (Fig 7) also exemplifies the importance of continuing AHF therapy for a sufficiently long time. He received AHF 2 days after the extraction, during which he showed no bleeding at all. On the fifth day he bled profusely from the socket of the tooth. After AHF however this bleeding topped. In the other patients the extractions were performed without complications. Judging from our experience the following regimen of treatment can be recommended for tooth extraction Adults should be given one dose of AHF before extraction half a dose 5—10 hours after the extraction, half a dose of AHF twice the next day and then 2 to 4 half-doses during the postoperative period (Fig 8) We also keep the patients in hospital one week after the extraction in order to be able to give AHF immediately in the event of bleeding symptoms. We usually extract only one tooth at a time, unless it is a question of neighbouring teeth.

The incision of the phlegmon in *A A fam 27 born 1941* (Fig 9) was complicated by bleeding on the fifth postoperative day The AHF level had by then decreased to 5 per cent. This shows that it is necessary to keep the AHF level high at least 10 days after surgical intervention on an infected wound. This is in agreement with the observations made by Macfarlane et al (51)

The other minor surgical interventions including circumcision for phimosis, reduction of bone fractures, skin biopsy and joint operations were performed under cover of AHF without bleeding complications. The cases reported under the heading of *haemorrhage after various surgical interventions* demonstrates the serious bleedings liable to occur if such operations are done without effective precautions against bleeding.

AHF was used for treatment of several *different bleeding episodes* in haemophiliacs. In all cases AHF produced effective haemostasis, and by daily injections normal haemostasis could be maintained for long periods It was sufficient to raise the AHF to 15—20 per cent of normal to control spontaneous haematomas and smaller spontaneous bleeding episodes.

We treated 83 episodes of *joint bleeding* The effect of fraction I—0 in these cases is difficult to evaluate. The only way to assess it would be to compare the results with previous

joint bleeding not treated with AHF in the same patient and to compare the state of the joints in patients who had regularly received tratment for *joint bleeding* with that of haemophilic relatives who have not received such therapy Fraction I—0 invariably gave prompt relief of pain and controlled the bleeding The haemarthrosis resolved much earlier than in previous episodes in these patients Nor did persistent joint deformities develop in these cases. No joint deformities have devoloped in those patients who have since 1956—1958 received fraction I—0 regularly during periods of joint bleeding Our experience hitherto thus suggests that if one to two doses of fraction I—0 are given in combination with immobilization of the joint, bleeding will stop Moreover subsequent early mobilization of the joint is necessary *preferably under* cover of AHF It is possible that this *might prevent development of dis*abling joint deformities On the other hand it appears *hardly* worth while using AHF for recurrent bleeding in already deformed joints *It* appears sufficient to raise the AHF level to about 20 per cent to prevent joint bleeding In *L B fam 72 born 1948* a haemarthrosis was aspirated We do not think that joint bleeding indicates puncture or that aspiration offers advantages over the treatment described above for joint bleeding Moreover puncture requires that the AHF content be held at a much higher level to prevent new bleeding from the wound *T S fam 32 born 1953* provides an example of the complications liable to occur in association with puncture without effective measures against bleeding (Fig 18)

In the patient *(L R C fam 71 born 1951)* with *intracranial haemorrhage the effect of* therapy with fraction I—0 was significant Nowadays, in order to prevent disasterous intracranial haemorrhage fraction I—0 is given prophylactically immediately after known injury to head

Gastrointestinal bleeding in haemophiliacs is a serious complication, and it may be equally dangerous in a patient with mild haemophilia We administered AHF to 13 patients with gastrointestinal bleeding Nine of these patients also received several transfusions of blood and fresh plasma, so that the effect of I—0 is difficult to evaluate, but all the patients recovered. In *M H., fam 27 born 1921* who had several episodes of severe gastrointestinal bleeding, it was not possible to control the bleeding by infusion of fresh blood and plasma *Administration of fraction* I—0 stopped the bleeding promptly and *appeared life-saving* (Fig 15)

We treated several cases with *haematuria After administration* of one or half a dose of fraction I—0 the *bleeding stopped. In some cases* the haematuria ceased in others, it recurred after 2 to 3 days to stop on repeated administration Several of the patients treated had previously had prolonged periods of haematuria refractory to blood transfusions *In K S., fam 45 born 1904* (Fig 2) the haematuria did not respond *to treat*ment with AHF Operation revealed the cause of the *bleeding, namely a* large renal aneurysm

We gave *AHF to 10 patients with retroperitoneal haemorrhage* A good clinical effect was *obtained in all* these cases and only few transfusions had to be given We feel that

in these cases it is necessary to increase the AHF to 30—60 per cent immediately in order to stop the bleeding promptly and then to maintain the AHF level between 15—30 per cent for 3—5 days. It was striking that in these cases fraction I—0 produced a very rapid improvement in the patients general condition. Retroperitoneal bleeding is a not uncommon complication in patients with severe haemophilia. *In R K., fam 88 born 1915* who had severe haemophilia A and an anti-AHF fraction I—0 had no effect and he died from a large retroperitoneal and intraabdominal haemorrhage.

We consider it extremely important in these cases of acute abdomen in haemophiliacs to treat the patients as if they had internal haemorrhage. If the symptoms respond to adequate treatment it suggests that the diagnosis is correct. If the abdominal symptoms persist and operation is necessary the coagulation defect must be controlled to enable operation. On analysis of Sköld's material (84) it was found that several patients died from bleeding in association with operation for supposed appendicitis which proved to be retroperitoneal bleeding Our cases of major surgery also included 2 in which laparotomy was done because of suspected appendicitis, but in which the course of the symptoms proved to be due to retroperitoneal bleeding.

Several episodes of *large haematomas* spontaneous or following injury were treated In these cases it is difficult to get an objective measure of the effect of therapy However no further bleeding ever occurred after administration of a whole or half a dose of AHF In addition the haematomas were rapidly resorbed. Several of these patients had had similar haematomas, which were not treated with AHF There was a marked difference in the rate of healing. In the cases of haematomas in the throat the effect of administration of AHF appeared to be directly life-saving. In spontaneous haematomas and haematomas following injury we feel it sufficient to keep the AHF level at 15—25 per cent.

We have treated 6 episodes of severe bleeding following different surgical operations (Figs 17 18) The effect of fraction I—0 in these cases was striking. In all the cases in which bleeding had persisted for a long time bleeding promptly stopped. In order to prevent continued bleeding and to secure healing, it was, however necessary to continue treatment with AHF for at least 7 days and to keep the AHF level at about 20 per cent It is obvious that if bleeding complications occur in the postoperative course despite a certain degree of healing, treatment must be started again as intensely as in connection with the original surgical procedures.

Prophylactic treatment with AHF has been tried in 3 cases Two haemophilic brothers, aged 15 and 13 years, with severe bleeding symptoms have received half a dose of fraction I—0 once a month prophylactically for 3 1/2 years. During this period the patients have been in good condition They have had bleeding episodes, but these have been less severe and less frequent than formerly The annual number of days in hospital was lower (Tables IX and X) The most striking effect was that the bleedings were not as severe as formerly After admini

stration of half a dose of fraction 1—0 the AHF rose to levels between 15—40 per cent The AHF content then fell fairly rapidly but the last few per cent only very slowly so that the AHF level during the course of the major part of the month was 1—3 per cent instead of 0 1 per cent or less, *i e* the level before prophylactic treatment was started In other words it appears that the haemophilia had changed from a severe to a moderate form Some of the bleeding episodes occurred when the interval between treatment was tentatively prolonged to 6 weeks or when the patient had received preparations of low activity The older boy has received the fraction 107 times within 5 years and no resistance to therapy has been observed The third boy who was treated prophylactically *(T B fam 140 born 1956)* had severe haemophilia A He has received the fraction prophylactically once a month since the age of 3 years He has had several episodes of bleeding which have however never been severe and he has not developed any deformities

It is of course, not possible to assess the value of prophylactic treatment of severe haemophilia A from these data Such evaluation would require *a much longer observation period* and a large number of cases as *a control group. Moreover the interval* between the injections of AHF *and the dosage of AHF must be varied* We nevertheless believe it well *worth while to continue* with these trials Prophylactic treatment of haemophilic *patients may be of* value in decreasing the frequency of severe *bleeding episodes*

Data on the survival of AHF varies. *Langdell Wagner* and Brinkhous (42) have observed a half-life time of 2—3 hours of AHF in haemophilic dogs A half life of 6—12 hours in haemophilic patients was found by Deutsch [cit Achenbach (1)] and Biggs (9) Half times of the order of 10 to 15 hours have been observed in human beings by McMillan et al (52) and by Pool and Robinson (74) McMillan et al (52) point out the existence of certain individuals with very rapid clearance rates. From our figures it is apparent that the consumption of AHF varies from one occasion to another Half times of the order of 10 to 20 hours predominate In a situation requiring coagulation of a large surface as in operations or injuries the consumption of AHF was much more rapid initially than when healing was complete or almost complete This is apparent from many of the cases reported (see Figs. 2, 3, 11 12) This has also been pointed out by Macfarlane et al (51) and by Kekwick and Wolf (39) In the presence of active haemorrhage the AHF also disappears more rapidly This is demonstrated *e g* in *T S fam 32 born 1953* (Fig 7) in whom the injected AHF disappeared more rapidly in connection with the acute bleeding episodes than when they were almost healed The AHF also disappeared more rapidly in patients with infections than in those treated prophylactically (Figs. 10 and 11) Macfarlane et al (51) pointed out that the cause of haemorrhage during treatment was either a failure to achieve an effective blood level of AHF or a decline of the blood level coinciding with further damage to the wound by trauma or infection Rapid consumption of AHF in connection with local infections for example was noted in

E. N. fam. 111 born 1929 and A. A. fam. 27 born 1941 (Figs. 9 and 17) In the presence of infection achievement of a satisfactory AHF level requires larger doses. In patients with fever and infections the AHF was consumed rapidly. This was apparent in several cases (Figs. 8 9 10 11) Excitation also appears to have an unfavourable effect on the rate of healing. This was obvious in *P. N. fam. 103 born 1915* (Fig. 8) It should be stressed that satisfactory haemostasis in haemophiliacs requires not only a certain level of AHF in the blood but also extremely careful and gentle surgical technique. In addition infections must be controlled and anaemia prevented and the patient should be kept quiet. Early immobilization is also of utmost importance.

Our studies suggest the usefulness of human fraction I—0 containing AHF for replacement therapy of most haemorrhagic episodes in classic haemophilia. Under cover of this fraction it is possible to perform safely even major surgical intervention.

Summary

Human fraction I—0 containing AHF (f. VIII) prepared by the glycine method of Blombäck and Blombäck has been used in Sweden since 1956 in the treatment of patients with haemophilia A. One dose of fraction I—0 is prepared from 1400 to 1600 ml of fresh normal citrated plasma. The mean *in vivo* yield of AHF in fraction I—0 in the best batches was about 100 per cent of normal plasma.

Fraction I—0 has now been given 818 times (497 whole doses) to 63 patients with haemophilia A, which was severe in 44 moderate in 12 and mild in 7. Forty six patients received repeated infusions of fraction I—0 and 11 patients 20 or more. One patient received 107 injections in the course of 5 years. No untoward reactions and no resistance to therapy have been observed. Two patients, however who received 118 and 65 infusions of fraction I—0 respectively developed hepatitis.

Major surgery was performed 8 times in 6 patients, of whom 3 had severe and 3 moderate haemophilia A (Table X) In connection with these operations 169 injections (116.5 doses) of fraction I—0 were given.

Minor surgery was performed 19 times on 12 patients, of whom 11 had severe and 1 moderate haemophilia (Table X) Injections of fraction I—0 were given 144 times (92 doses)

Bleeding episodes such as haemarthrosis, haematoma, retroperitoneal and gastrointestinal haemorrhage, haematuria, intracranial haemorrhage and haemorrhage after surgery were treated in 60 patients (101 bleeding episodes) (Table X) Fraction I—0 was given 426 times (243 whole doses)

After injection of fraction I—0 abnormal bleeding was arrested and surgical procedures could be performed without any haemorrhagic complications, provided the AHF level was kept at a sufficiently high level. In order to control severe bleeding episodes it was necessary to increase the AHF level of the patient to 30—50 % of normal immediately and then to maintain it at about 20—30 % until healing was complete. In surgery the plasma AHF level was kept at 30—0 % during the operation and then at about 20—40 % in the postoperative

period For management of haemorrhages such as joint bleeding haematoma and haematuria, an AHF level of 10—20 % appeared sufficient The advantages of therapy with fraction I—0 in haemarthrosis and spontaneous haematoma are difficult to evaluate

Prophylactic treatment with half a dose of fraction I—0 once a month has been given to 3 boys with severe haemophilia A for 2 to 3 1/2 years. During the prophylactic treatment period the frequency and severity of bleeding episodes had diminished

Our studies suggest that human fraction I—0 is valuable in replacement therapy in haemophilia A

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From the Chemistry Department II (Head: Erik Jorpes, M.D.) Karolinska Institutet, Stockholm and the Department of Medicine I (Head: Erik Skald, M.D.), S:t Eriks Sjukhus, Stockholm, Sweden.

A CLINICAL AND MEDICO SOCIAL STUDY OF HAEMOPHILIA IN SWEDEN

By

OLOF RAMGREN

DISSERTATION

KAROLINSKA MEDICO-KIRURGISKA INSTITUTET

STOCKHOLM 1962

This investigation was suported by grants from the Swedish Medical Research Council to Docent Erik Sköld Stiftelsen Terese och Johan Anderssons Minne Eva och Oscar Ahréns Stiftelse, Karolinska Institutets Reservationsanslag and Research Grant No H-4295 (R1 C1—2) to Professor Erik Jorpes from the National Heart Institute National Institutes of Health U.S. Public Health Service

This dissertati n is a discussion and summary of the following six papers

I. NILSSON I. M., BLOMBÄCK, M. AND RAMGREN O
H emophilia in Sweden. I Coagulation studies. A ta Med. Scand. *170* 665—682, 1961.

II. NILSSON I. M., BLOMBÄCK. M., RAMGREN O AND VON FRANCKEN I :
Haemophilia in Sweden. II. Carriers f Haemophilia A and B. Acta Med. Scand. *171* 223—235, 1962.

III. R MGREN O
Haemophilia in Sweden. III. Symptomat logy with special reference to differences between haemohpili A and B. Acta Med. Scand. *171* 237—242, 1962.

IV. RAMGREN O NILSSON I. M. AND BLOM ÄCK, M.
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VI. NILSSON, I. M., BLOM ÄCK. M. AND RAMGREN O
Haemophilia i Sweden. VI Treatment of haemophilia A with the human antihaemophilic factor preparation (fraction I—0) Acta Med Scand. *171* Supplementum 379 pp 61—110 1962.

R f rence will be made t these papers using the Roman figures as listed above

THE SYMBOLS USED IN THE PEDIGREES

NORMAL WOMAN

NORMAL WOMAN CHILDLESS

FEMALE GENETIC CARRIER

FEMALE GENETIC CARRIER, EXAMINED BY COAGULATION TESTS SEE PAPER II.

FEMALE CARRIER ACCORDING TO COAGULATION TESTS, SEE PAPER II

FEMALE HAEMOPHILIAC

NORMAL MAN

HAEMOPHILIAC

HAEMOPHILIAC, EXAMINED BY COAGULATION TESTS, SEE PAPER I.

PROBABLE HAEMOPHILIAC

DESCENDANTS, NUMBER AND SEX UNKNOWN OR OF NO INTEREST

V-9 GENERATION V MEMBER NO. 9 AS COUNTED FROM THE LEFT

DESCENDANTS DESIGNED AS ◇ ARE NOT COUNTED

An international committee (organized in Basle in 1954) has devoted particular interest to the nomenclature of blood clotting factors. Both AHF and AHG have been used to denote the antihaemophilic factor (factor VIII). In our earlier publications, we have used AHG, whereas in the present paper it has been replaced by AHF. The reason for this change is twofold. Firstly, since the exact chemical nature of the antihaemophilic factor is unknown, the "G" standing for globulin is based only on a supposition. Consequently "F" denoting "factor" is more correct, as well as being in conformity with the recommendations of the Nomenclature Committee (10). Secondly AHG also stands for antihuman globulin, the antiserum used in the Moreschi—Coombs test. Since both the antihaemophilic factor and the antihuman globulin preparations are stored at blood transfusion centres, there is a risk of confusing these two preparations. Moreover the antihuman globulin was first described by Moreschi (8) in 1908 and therefore has a prior claim to the abbreviation AHG.

Introduction

The early history of haemophilia, its inheritance symptomatology coagulation defects and treatment have been extensively reviewed in Scandina ia by Andreassen (1) 1943, Sköld (15) 1944 Blombäck (3) 1958 Ikkala (7) and Sjolin (14) 1960 in the German literature by Grandidier (6) 1877 and Schlossman (13) 1930 and in the Anglo-Saxon literature by Quick (10 11) 1942 and 1957 Biggs & Macfarlane (2) 1953 Brinkhous (5) 1957 and Ratnoff (12) 1960

New concepts on the pathogenesis of haemophilia were introduced by the finding of Pavlovsky (9) in 1947 that plasma from one haemophiliac could correct the clotting defect in the blood from another This aroused fresh interest in the study of haemophilia. In 1960 reviews on haemophi lia in Denmark and in Finland were published by Sjolin (14) and by Ikkala (7) respectively

A review on *haemophilia in Sweden* was published by Sköld (15) in 1944 He investigated 60 families with 101 living haemophiliacs His aim was to follow the hereditary pattern in the Swedish families, to study the symptomatology and coagulation time in whole blood both in the haemophl liacs and in the carriers, as well as to evaluate the effect of blood transfusion therapy He organized blood transfusion centres at regional hospital throughout the country and put the haemophiliacs in touch with these hospitals. Special schools and vocational training were organized for haemophiliacs at the institutions for handicapped persons, and attempts were made to minimize the number of haemophiliacs by information about birth-control and by sterilization of both haemophiliacs and carriers.

In 1954 investigations with the object of preparing a stable fibrinogen preparation were started at the Chemistry Department II Karolinska Institutet Stockholm. A stable fibrinogen fraction, denoted as fraction I—0 (3) was obtained, and was shown to contain a high content of AHF (factor VIII) The fraction was therefore used for treatment of haemophilia A and von Willebrand s disease (3) This initiated a new interest in the study of haemophilia in Sweden.

The present investigation was started in 1955 as a follow-up of Sköld s aforementioned study (15) Special interest was focused on the coagulation status of the haemophiliacs and carriers (Papers I and II) the symptomatology of haemophilia A and B was compared (Paper III) hereditary in estigations were undertaken (Paper IV) the medico-social aspects of haemophilia in Sweden were penetrated (Paper V) and the treatment of haemophilia A with human AHF preparations was evaluated (Paper VI) The results will be summarized and discussed in the following

Methods and clinical material

The investigations were started as a follow-up of Sköld's aforementioned study (15) and his 60 haemophilic families were re-investigated Unpublished data on these families were kindly placed at my disposal by E. Sköld.

In order to trace new haemophiliacs, the hospital reports to the Royal Swedish Medical Board for the years 1912—1957 were surveyed and the

Table I New haemophilic families detected during 1961

Haemophilia Type	No. of families	No. of haemophiliacs	Born years					
			1921	1921–1940	1941–1950	1951–1955	1956–1960	1961
A severe	6	7	—	2	—	—	4	1
A moderate	2	3	1	—	—	1	1	—
A mild	8	8	2	2	3	1	—	—
B severe	1	1	—	—	1	—	—	—
B moderate	—	—	—	—	—	—	—	—
B mild	1	1	—	—	—	1	—	—
Total	18	20	3	4	4	3	5	1

available case records were examined The haemophiliacs (or when more suitable their relatives) received a questionnaire about the family relationships, and further information about the families was obtained from the parish registers A clinical examination was made of most of the patients, either by the author or by one of the collaborators (M Blombäck and I M Nilsson) and a questionnaire regarding the clinical symptoms and medico-social consequences of their haemophilia was answered by all but a few living haemophiliacs

Coagulation studies of haemophiliacs or carriers included determinations of the coagulation time factor VIII and factor IX prothrombin consumption test bleeding time platelet counts prothrombin + proconvertin (factor II + VII) factor V fibrinogen and circulating anticoagulants. A coagulation status for a haemophiliac had all but one or two of these determinations, whereas a carrier was usually investigated only by the four first mentioned tests. Details of the methods are given in Paper I

A short case history was compiled for each haemophiliac, as well as a pedigree of the family They are given on pages 117–181 in this paper

Altogether 180 haemophilic families in Sweden with 253 living haemophiliacs were investigated

The coagulation studies of haemophiliacs and carriers, and investigations of the symptomatology hereditary pattern and medico-social aspects were continued until December 31 1960 The survey of treatment with the AHF preparation was carried as far as August 31 1961 During 1961 an additional 20 haemophiliacs belonging to 18 new families, were diagnosed (Table I) One severe haemophiliac (case IV 6 family 147) was born in the 180 families covered in the present survey

The patients were classified as severe moderate or mild cases of haemophilia A and B respectively according to the plasma AHF or B factor level as recommended by Brinkhous & Graham (4) *i e.*,

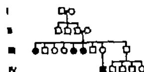
severe haemophilia A and B AHF or B factor < 1 per cent of normal

moderate haemophilia A and B AHF or B factor 1—4 per cent of normal

mild haemophilia A and B AHF or B factor 5—25 per cent of normal.

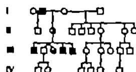
The patients not classified by coagulation studies were assigned to the groups of clinically severe or mild haemophilia as judged by the clinical features and coagulation time

Family 1, Haemophilia, clinically severe form.



IV 1 (Sköld I: 1) (A.E.) Born 1895, died 1923 of haemophilia. First symptom of bleeding at th age of 7 months (nasal haemorrhages) hospitalized several times for haematomata and haemarthrosis. Worked as a shoemaker Died in mental hospital.

Family 2, Haemophilia, clinically severe form.



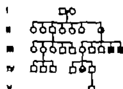
I 2 I said t have had some "strange disease showing itself in boils.

III 1 (Sköld II 1) (S.E.) Died at two years of age of bleeding from a ruptured frenulum of the upper lip.

III: 4 (Sköld II 4) Died at 10 months of age of bleeding.

III 5 (Sköld II 5) (R.E.) Born 1916, died 1940 f internal bleeding (peritoneal and retroperitoneal) First symptom of haemophilia at ne month of age (excessive hae morrhage from a cut wound) Hospitalized thirteen times for bleedings from tooth extractions, haemarthrosi and ha maturia. H had no occupation.

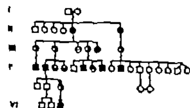
Family 3, Haemophilia, clinically severe form.



III 9 (Sköld II 9) (J.B.) Born 1903, d ed 1933 of complications after bleeding f om the nose. First symptom of haemophilia t one year of age (subcutaneous haemorrhages) Hospitalized several times for joint bleedings (o a stiff knee) bleeding after tooth xtraction and nosebleed. Worked as a journalist.

III 10 (Sköld II 10) Died after birth of bleeding.

Family 4, Haemophilia, A, severe form.



IV 1 (Sköld II 1) (E.J) Born 1883 died 1886 of haemophilia, repeated haemorrhages from the nose.

IV 2 (Sköld II: 2) (E.P) Born 1887 died 1934 of gastrointestinal hae morrhage.

IV 6 (Sköld II 6) (N.H.) Born 1901 died 1915 o gastrointestinal hae-

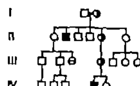
morrhage *Treated several times* in hospital for joint, renal and gastrointestinal haemorrhages. Worked on his father's farm.

IV 8 (Sköld II 8) (G.H.) Born 1907 died 1956 of renal haemorrhage. Treated several times in hospital for haemarthrosis and haematomata in the muscles. Worked as a farmer

IV 10 (Sköld II 10) (J.B.) Born 1889 died 1907 of haemophilia re peated bleedings from various or gans and joints.

VI 3 (C.H.N.) Born *1957 First symptom of haemophilia at four* months of age (subcutaneous haemorrhage) He has bled from the lips after traumata. Haemarthrosis a few times, has received about *10 blood transfusions.*

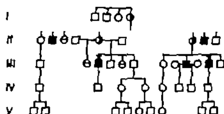
Family 5, Haemophilia, *clinically severe form.*



II 2 (Sköld III 2) (N.H.) Born about 1880 died at early age of excessive haemorrhage.

IV 4 (Sköld I 4) (J.E.) Born 1936, died *1943 of gastrointestinal haemorrhage. First symptom of haemophilia* was a renal haemorrhage at the age of five months. Hospitalized about ten times for haemorrhages *from wounds and haematomata* in muscles. Repeated gastrointestinal haemorrhages.

Family 6, Haemophilia A, mild form.



II 2 (Sköld IV 4) *(K.N.) Born 1863,* died 1885 of haemophilia.

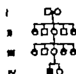
II 6 (Sköld IV 7) *(J.N.) Born 1872.* Died of haemophilia in youth.

III 3 (Sköld III 3) *(B L.) Born 1903.* No symptoms of haemophilia during childhood Repeated haemorrhages after tooth extractions. No joint bleedings. Free from mili*tary service on account of haemophilia* Is a department head in a business firm.

III 8 (Sköld III 7) (E.A.) Born *1895* died 1935 of haemorrhages from tonsillitis. Severe bleeding after tooth extraction. No joint bleed*ing.*

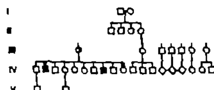
III 9 (Sköld III 8) (H.A.) Born *1899 First symptom of haemophilia at* the age of 30 years (excessive haemorrhage after operation) Repeated bleedings after wounds and ones a renal haemorrhage *No joint bleedings.* In 1928 appendectomy bled severely after and received three blood trans fusions. *Works as an electrician.*

Family 7 Haemophilia, clinically severe form.



IV 1 (Sköld I:1) (A.A.) Born 1924, died 1947 of haemorrhage. First symptom of haemophilia at one year of age (excessive haemorrhages after a cut wound) Repeated severe haemorrhages from wounds, haemarthrosis, and haematomata in muscles. At 1935 he had a minor cerebral haemorrhage, recovered completely Hospitalized several times for wound and renal haemorrhages. Worked as as waiter

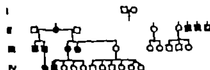
Family 8, Haemophilia B, severe form.



IV 2 (Sköld II 2) (K.E.C.) Born 1909 First symptom of haemophilia before one year of age (subcutaneous haemorrhages) Repeated haemarthroses, which have caused impaired function of the knee, elbow and ankle joints. Hospitalized several times for gastrointestinal haemorrhages and has received 7 blood transfusions. Free from military service No occupation, lives on disablement pension.

IV 8 (Sköld II 8) (A.C.) Born 1922, died 1959 of gastrointestinal haemorrhage. First symptom of haemophilia at 8 months of age (subcutaneous haemorrhages) Repeated haemarthroses a d haemorrhages from wounds. He had no profession and lived on disablement pension.

Family 9 Haemophilia A, moderate form.

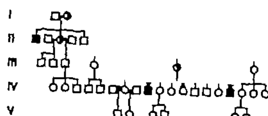


III 1 (Sköld I:1) (X.F) Born 1908, died 1913 in gastrointestinal bleeding. Had always bled easily

III 2 (Sköld I 2) (E.F) Born 1911 First ymptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemorrhages in the knee and elbow joints, which has caused a stiff right knee and impaired function of the left knee and of the elbows. H has received a few blood transfusions. Works as a tailor

IV 2 (S.M.) Born 1943. First symptom of haemophilia at one and a half years of age (excessive haemorrhage after cut wound) He has had haemarthroses about 20 times in knee and elbow joints, without causing impaired function. He has received four blood transfusions. He has been able to attent school regulary and be alms to attend a business school in order to get office work.

Family 10, Haemophilia A, severe form.



II 1 (Sköld III 1) Born 1870 died 1877 of croup Is said to have had bleeding tendency

IV 7 (Sköld I 6) (Y.A.) Born 1935 First symptom of haemophilia at about 2 years of age (subcutaneous haemorrhages) Repeated haemorrhages in all the main joints, especially the knees, which are booth stiff Hospitalized several times and has received about 45 blood transfusions. He works as a chemical engineer and is free from military service.

IV 14 (R.A.) Born 1953 First symptom of haemophilia at about one year of age (subcutaneous haemorrhages) Repeated haemorrhages in the main joints, which has caused impaired function of both knees. He attends a special school for disabled children In 1960 he was appendectomized and treated with AHF preparation He has received about 5 blood transfusions.

Family 11 Haemophilia A, severe form.

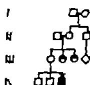


III 2 (Sköld I 1) (B.L.) Born 1937 First symptom of haemophilia at 2 years of age (excessive haemorrhage from a wound in the upper lip) Repeated haemarthroses in the main joints. Both knees have impaired movement which renders difficulty in walking down stairs. Hospitalized several times for haemarthrosis and renal haemorrhage, and has received about 10 blood transfusions. Free from military service. He is a factory worker

III 3 (Sköld I 2) (S.L.) Born 1938, died 1946 of haemorrhage after an auto accident. First symptom of haemophilia at two years of age (excessive haemorrhage from wound in the mouth) He always bled easily after wounds and had repeated haemarthroses.

III 4 (K.L.) Born 1944 First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemarthroses. After a haematoma in the back he has difficulties walking normaly and cannot raise the right foot. Hospitalized several times for haematomata and haemarthrosis. In 1918 he had a subarachnoidal haemorrhage from which he recovered completely He has received about 30 blood transfusions. He has been able, in spite of several hospitalization periods, to continue his studies normally

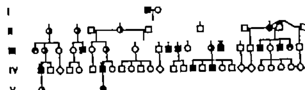
Family 12, Haemophilia A, severe form.



IV 3 (Sköld I 3) (T.B.) Born 1931 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) Repeated haemarthroses have impaired the function of the knees, confining him to a wheel-

chair. Hospitalized several times for joint bleedings and renal haemorrhages. He has received about 100 blood transfusi ns. Received education in the home during the last two school years and has since received special vocational trai ing. Works now as a draughtsma in an engineering office. Free from military service.

Family 13, Haemophilia A, mild form.



I 1 (Sköld IV: 1) (A.D.) Died of haemophilia at bout 50 years of age.

III 5 (Sköld II 12) (B.N.) Died of cerebral haemorrhage t 30 years of age.

III 10 (Sköld II 5) (E.H.) Born 1916, died 1918 of haemophilia.

III 11 (Sköld II 6) (L.H.) Born 1919 First ymptom of haemophilia at the age of five years (excessive ha morrhage from a cut wound) Repeated haemarthroses but o impaired joi t function. Treated i hospital several times and has received about 50 blood transfusions. Free from military service. Work as a painter

III 11 (Sköld II 9) (N.O.H.) Born 1921. First sympt m of ha mophilia t the ge of five years (nasal haemorrhages) Repeated haemar throses especially in the ankle joints, which have slightly impaired functi n. Hospitalized 25 times for gastrointestinal haemorrhages nd has received about 100 blood transfusions. Free from military service. No occupation, lives on disablement pen ion.

III 17 (Sköld II 14) (T.L.) Born 1910. A few times copious gastrointestinal haemorrhages, no joint bleedings. Works a railwayman.

III 19 (Sköld II 16) (T.X.L.) Born 1913, died 1916 of gastrointestinal hae morrhage. During life repeated gastrointestinal haemorrhages.

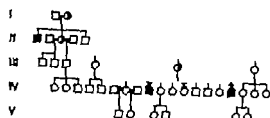
IV 1 (K.E.T.) Born 1923. First symptom of haemophilia at two years f ag (subcutaneous haemorrhages) Only one bleeding in the hip joint. Hospitalized 12 times for gastroi testinal and once for renal haemorrhage. He has received about 50 blood transfusions. Free from military service. H is a factory worker

IV 6 (Sköld I 1) (G.J.) Born 1931 First ymptom of haemophilia at 1 ½ years of age (haemathrosis in the knee) Hospitalized ten times for gastroi testin l haemorrhages. H has recei ed about 30 blood transfusions. Free from military service Repeated haemarthrosis but no impaired joint function. Works as a painter

IV 14 (B.F.) Born 1911. First symptom of haemophilia at two years of age (subcutaneous haemorrhage) \ haemarthrosis.

IV 16 (K.F.) Born 1947 died 1948 of haemophilia.

Family 10, Haemophilia A, severe form.



II 1 (Sköld III 1) Born 1870 died 1877 of croup Is said to have had bleeding tendency

IV 7 (Sköld I 6) (Y.A.) Born 1935 First symptom of haemophilia at about 2 years of age (subcutaneous haemorrhages) Repeated haemorrhages in all the main joints, especially the knees, which are booth stiff Hospitalized several times and has received about 45 blood transfusions. He works as a chemical engineer and is free from military service

IV 14 (R.A.) Born 1953. First symptom of haemophilia at about one year of age (subcutaneous haemorrhages) Repeated haemorrhages in the main joints, which has caused impaired function of both knees. He attends a special school for disabled children. In 1960 he was appendectomized and treated with AHF-preparation He has received about 5 blood transfusions.

Family 11, Haemophilia A, severe form.

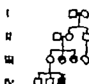


III 2 (Sköld I 1) (B.L.) Born 1937 First symptom of haemophilia at 2 years of age (excessive haemorrhage from a wound in the upper lip) Repeated haemarthroses in the main joints. Both knees have impaired movement which renders difficulty in walking down stairs. Hospitalized several times for haemarthrosis and renal haemorrhage, and has received about 70 blood transfusions. Free from military service. He is a factory worker

III 3 (Sköld I 2) (S.L.) Born 1938, died 1946 of haemorrhage after an auto accident. First symptom of haemophilia at two years of age (excessive haemorrhage from wound in the mouth) He always bled easily after wounds and had repeated haemarthroses.

III 4 (K.L.) Born 1944 First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemarthroses. After a haematoma in the back he has difficulties walking normaly and cannot raise the right foot. Hospitalized several times for haematomata and haemarthrosis. In 1918 he had a subarachnoidal haemorrhage from which he recovered completely He has received about 30 blood transfusions. He has been able in spite of several hospitalization periods to continue his studies normally

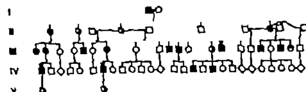
Family 12, Haemophilia A, severe form.



IV 3 (Sköld I 3) (T.B.) Born 1934 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) Repeated haemarthroses have impaired the function of the knees, confining him to a wheel

chair. Hospitalized several times for joint bleedings and renal hae morrhages. H has received about 100 blood transfusions. Received education in the home during the last two school years and has since received special vocational trai i g. Works now as a draughtsman in an ngineering office. Free from military service.

Family 13, Haemophilia A, mild form.



I 1 (Sköld IV 1) (A.D.) Died of haemophilia at about 50 years of age.

III 5 (Sköld II 12) (R.N.) Died of cerebral haemorrhage at 30 years of age.

III 10 (Sköld II 5) (E.H.) Born 1916, died 1918 of haemophilia.

III 11 (Sköld II 6) (L.H.) Born 1919. First symptom of haemophilia at the ge of five years (excessive haemorrhage from a cut wound). Repeated haemarthroses but no impa red joint function. Treated in hospital several times and has recei ed about 50 blood transfusions. Free from military service. Works as a painter.

III 14 (Sköld II 9) (N.O.H.) Born 1924. First symptom of haemophilia at the ge of five years (nasal haemorrhages). Repeated haemar throses especially in the ankle joints, which he slightly impaired function. Hospitalized 25 times for gastrointestinal haemorrhages nd has received about a 100 blood transfusions. Free from military service. No occupation, lives on disablement pension.

III 17 (Sköld II 14) (T.L.) Born 1910. A few times copious gastrointestinal haemorrhages, no j int bleedings. Works as a railwayman.

III 19 (Sköld II 10) (T.X.L.) Born 1913, died 1946 of gastrointestinal haemorrhage. Duri g lif repeated gastrointestinal haemorrhages.

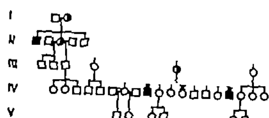
IV 1 (K.E.T.) Born 1923. First symptom of haemophilia t tw years of age (subcutaneous haemorrhages). Onl one bleeding in the hip joint. Hospitalized 12 times for gastrointestinal and once for renal haemorrhage. He has received about 50 blood transfusions. Free from military service. He is a factory worker.

IV 6 (Sköld I 1) (G.J.) Born 1931. First symptom of haemophilia at 1 ½ years of age (haemathrosis in the knee). Hospitalized ten times for gastrol testinal haemorrhages. He has received about 30 blood transfusions. Free from military service. Repeated haemarthrosis but no impaired joint function. Works as a painter.

IV 14 (B.F.) Born 1944. First symptom of haemophilia t two years of age (subcutaneous haemorrhage). No haemarthrosis.

IV 16 (K.F.) Born 1947 died 1948 of haemophilia.

Family 10, Haemophilia A, severe form.



II 1 (Sköld III 1) Born 1870 died 1877 of croup Is said to have had bleeding tendency

IV 7 (Sköld I 6) (S.A.) Born 1935 First symptom of haemophilia at about 2 years of age (subcutaneous haemorrhages) Repeated haemorrhages in all the main joints, especially the knees, which are booth stiff Hospitalized several times and has received about 45 blood transfusions. He works as a chemical engineer and is free from millitary service

IV 14 (B.A.) Born 1953. First symptom of haemophilia at about one year of age (subcutaneous haemorrhages) Repeated haemorrhages in the main joints, which has caused impaired function of both knees. He attends a special school for disabled children In 1960 he was appendectomized and treated with AHF-preparation He has received about 5 blood transfusions.

Family 11, Haemophilia A, severe form.

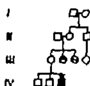


III 2 (Sköld I 1) (B.L.) Born 1937 First symptom of haemophilia at 2 years of age (excessive haemorrhage from a wound in the upper lip) Repeated haemarthroses in the main joints. Both knees have impaired movement which renders difficulty in walking down stairs. Hospitalized several times for haemarthrosis and renal haemorrhage, and has received about 70 blood transfusions. Free from millitary service He is a factory worker

III 3 (Sköld I 2) (S.L.) Born 1938, died 1946 of haemorrhage after an auto accident. First symptom of haemophilia at two years of age (excessive haemorrhage from wound in the mouth) He always bled easily after wounds and had repeated haemarthroses.

III 4 (K.L.) Born 1944 First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemarthroses. After a haematoma in the back he has difficulties walking normaly and can not raise the right foot. Hospitalized several times for haematoma and haemarthrosis. In 1958 he had a subarachnoidal haemorrhage from which he recovered completely He has received about 30 blood transfusions. He has been able in spite of several hospitalization periods, to continue his studies normally

Family 12, Haemophilia A, severe form.



IV 3 (Sköld I 3) (T.B.) Born 1931 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) Repeated haemarthroses have impaired the function of the knees, confining him to a wheel

IV 7 (Sköld II: 7) (E.A.) Born 1917 died 1942 of gastrointestinal haemorrhage. First symptom of haemophilia at on year of age (subcutaneous haemorrhage) Repeat ed haemarthrosis, which caused severe disability with contractures in the knees. Hospitalized several times for renal and gastrointestinal haemorrhages. He was blind and had no occupation.

IV 9 (Sköld II 9) (H.L.) Born 1901, died 1930 of cerebral haemorrhage after an uto accident.

IV 12 (Sköld II 12) (U.L.) Born 1917 died 1938 of gastrointestinal haemorrhage. Repeated haemarthrosis and gastrointestinal haemorrhages. Received about 15 blood transfusions.

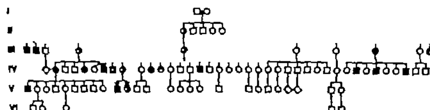
V 5 (Sköld I 5) (F.B.) Born 1928, died 1943 of interior bleedi g after an abdominal haematoma.

Family 17, Haemophilia A, severe form.



III 1 (J.A.) Born 1934. First symptom of haemophilia at tw years of age after a wound in the upper lip Repeated haemarthrosis, which has caused impaired function of the knee and elbow joints. Hospitalized several times for joint, renal and gastrointestinal bleed i gs, and has received about 40 blood transfusions. He has attended a special school for disabled children and is an educated draughtsman but works as a con troller

Family 18, Haemophilia A, severe form.



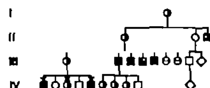
IV 8 (Sköld II 11) (G.B.) Born 1912. First symptom of haemophilia at one yea of age (subcutenous haemorrhages) Repeated haemarthrosis which has caused impaired functions of the knee and lbow joints. Hospitalized se er l times for haemarthroses, gastrointesti nal nd ren l haemorrhages. He has successfully been operated for ppendicitis, tonsillitis and illm. He has recei ed about 150 blood transfusion. le could attend school normally and was free from military service. He works as an attendant at a bus station.

IV 15 (Sköld II 19) (W.M.) Born 1914 died 1955 of haemorrhages after a abdominal operation. He had repeated haemarthrosis and was disabled. He worked as an attend ant at a small shop.

IV 31 (Sköld II 36) (X.A.) Died of haemophilia in youth.

IV 32 (Sköld II 23) (X.X.A) Died of haemophilia in youth.

Family 14, Haemophilia B, moderate form.



II 3 (Sköld III 3) (S.K.) Born 1867 died 1886 of intracranial haemorrhage

III 2 (Sköld II 2) (G.S.) Born 1895 died 1946 of interior haemorrhage (infected haematomata) Repeated haemarthrosis. Worked as a farmer

III 3 (Sköld II 3) (H.S.) Born 1897 died 1931 of cerebral haemorrhage. Severely disabled

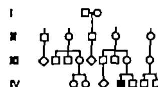
III 4 (Sköld II 4) (K.S.) Born 1899 died 1937 of gastrointestinal haemorrhage

III 5 (Sköld II 5) (E.S.) Born 1901 died 1903 of haemophilia.

IV 1 (Sköld I 1) (B.G.) Born 1922. First symptom of haemophilia at three years of age (haemarthrosis) Repeated haemarthroses in the main joints, which have impaired the function of knees and elbows. Hospitalized a few times and has received about 20 blood transfusions. Free from military service Works as a tailor and has disablement pension.

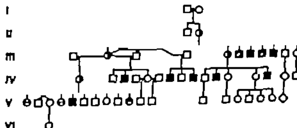
IV 5 (Sköld I 4) (S.G.) Born 1928. First symptom of haemophilia at three years of age (haemarthrosis) Repeated haemarthroses in the main joints, which have impaired the function of knees and elbows. Never hospitalized and has not received blood transfusions. Free from military service Works as a tailor and has disablement pension

Family 15, Haemophilia, clinically severe form.



IV 3 (Sköld I 4) (B.P) Born 1930 died 1956 of haemorrhage after an accident. First symptom of haemophilia at two years of age (excessive haemorrhage from a wound in the lip) Repeated haemarthrosis which impaired the functions of the main joints. Free from military service. No occupation.

Family 16, Haemophilia, clinically severe form.



III 3—6 (Sköld III 4—7) Died of bleedings in youth.

IV 3 (Sköld II 3) (X.A.) Died of interior haemorrhage in youth.

IV 5 (Sköld II 5) (X.X.A.) *Born 1912* died 1915 of *bleeding from a* wound

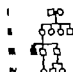
IV 7 (Sköld II 7) (E.A.) Born 1917 died 1948 of gastrointestinal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous ha morrhage) Repeated haemarthrosis, which caused severe disability with contractures in the knees. Hospitalized several times for renal and gastrointestinal haemorrhages. He was blind and had no occupation.

IV: 9 (Sköld II 9) (H.L.) Born 1903, died 1930 of cerebral haemorrhage after an auto accident.

IV: 12 (Sköld II 12) (U.L.) Born 1917 died 1938 of gastrointestinal haemorrhage. Repeated haemarthrosis and gastrointestinal haemorrhages. Recei ed about 15 blood transfusions.

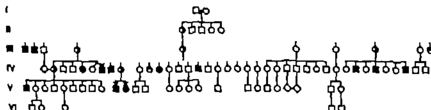
V 5 (Sköld I 5) (F.B.) Born 1928, died 1943 of interior bleeding after an abdominal haematoma.

Family 17 Haemophilia A, severe form.



III 1 (J.A.) Born 1934. First symptom of haemophilia at two years of ge after a wound in the upper lip Repeated haemarthrosis, which has caused impaired function of the knee and elbow joints. Hospitalized several times for joint, renal and gastrointestinal bleedings, and has received about 40 blood transfusions. He has attend ed a special school for disabled children and is an educated draughtsman but works as a controller

Family 18, Haemophilia A, severe form.



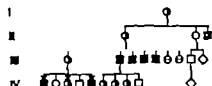
IV 9 (Sköld II 11) (G.B.) Born 1912. First symptom of haemophilia at one yea of ag (subcutenous haemorrhages) Repeated haemarthrosis which has caused impaired functions of the knee and lbow joints. Hospitalized se eral times for haemarthroses, gastrointesti l and renal haemorrhages. He has successfully been operated for ppendicitis, tonsillitis and filus. He has received about 150 blood transfusion. He could attend school normally and was free from military service. He works as an ttendant at a bus station.

IV 18 (Sköld II 19) (W.J.L.) Born 1914, died 1955 of haemorrhages after an abdominal operation. He had repeated haemarthrosis and was disabled. He worked as an attendant at a small shop.

IV 31 (Sköld II 36) (X.A.) Died of haemophilia in youth.

IV 33 (Sköld II 38) (X.X.A) Died of haemophilia i youth.

Family 14, Haemophilia B, moderate form.



II 3 (Sköld III 3) (S.K.) Born 1867 died 1880 of intracranial haemorrhage.

III 2 (Sköld II 2) (G.S) Born 1895 died 1946 of interior haemorrhage (infected haematomata) Repeated haemarthrosis. Worked as a farmer

III 3 (Sköld II 3) (H.S.) Born 1897 died 1931 of cerebral haemorrhage Severely disabled.

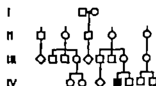
III 4 (Sköld II 4) (K.S.) Born 1899 died 1937 of gastrointestinal haemorrhage.

III 5 (Sköld II 5) (E.S.) Born 1901 died 1903 of haemophilia.

IV 1 (Sköld I 1) (B.G.) Born 1922. First symptom of haemophilia at *three years of age (haemarthrosis)* Repeated haemarthroses in the main joints which have impaired the function of knees and elbows. Hospitalized a few times and has received about ?0 blood transfusions. Free from military service Works as a tailor and has disablement pension.

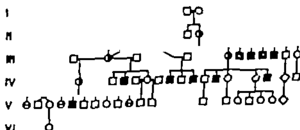
IV 5 (Sköld I 4) (S.G) Born 1928. First symptom of haemophilia at three years of age (haemarthrosis) Repeated haemarthroses in the main joints, which have impaired the function of knees and elbows. Never hospitalized and has not received *blood transfusions.* Free from military service. Works as a tailor and has disablement pension

Family 15, Haemophilia, clinically severe form.



IV 3 (Sköld I 4) (B.P) Born 1930 died 1956 of haemorrhage after an accident. First symptom of haemophilia at two years of age (excessive haemorrhage from a wound in the lip) Repeated haemarthrosis which impaired the functions of the main joints. Free from military service No occupation.

Family 16, Haemophilia, clinically severe form.

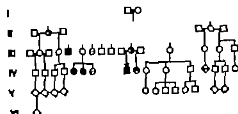


III 3—6 (Sköld III 4—7) Died of bleedings in youth.

IV 3 (Sköld II 3) (X.A.) Died of interior haemorrhage in youth.

IV 5 (Sköld II 5) (X.X.A) Born 1912, died 1915 of bleeding from a wound

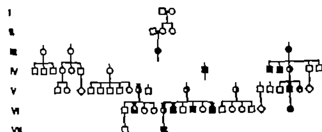
Family 21, Haemophilia A, severe form.



III 3 (Sköld IV:3) (A.F) Born 1861 died 1924 of internal haemorrhage. He had repeated haemarthrosis and had difficulty in walking.

IV 8 (Sköld III 5) (C.F) Born 1900 First symptom of haemophilia at one year of age (haemarthrosis) Repeated haemarthroses, which have caused impaired function f the ankle knee shoulder and elbow joints. Hospitalized numerous times for haemarthrosis and renal haemorrhages and has received over 500 blood transfusions. He worked as a book-binde but is now severely disabled and lives on disablement pension.

Family 22, Haemophilia A, severe form.



IV 8 (A.F) Born 1888, died 1932 of gastrol testinal haemorrhage.

IV 9 (Sköld IV 8) (X.S.) Born 1883, died of bleeding after few days.

IV 10 (Sköld IV 9) (J.S.) Born 1883, died 1906 of gastrointestinal haemorrhage He had repeated ha marthrosis.

V 16 (Sköld I 5) (P.F) Born 1914 First symptom f haemophilia 1/ years old (subcutaneus haemorrhage) Repeated haemarthroses, which has caused stiff ess in both knees. Hospitalized several times for haemar throsis, gastrointestinal and renal haemorrhages. Treated with profylacti blood transfusions duri g the years 1913—1937 d has received about 200 blood transfusions. He attended ordinary school and works as a clerk.

VI 2 (B.E.) Born 1948. First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemarthroses, which have caused impaired functi n of the left knee joint. H can atte d ordinary school.

VI 6 (R.K.) Born 1910 First symptom of haemophilia t 6 mo ths of age (subcutaneous haemorrhages) Repeated haemarthroses, which have impaired the function of the elbows. Hospitalized several times for haemarthrosis d once for gastrointestinal haemorrhage, and has received about 10 blood transfusions. H has been able to t tend ordinary school. Free from military service. He works part time as clerk in his f ther' firm, but has disablement pension.

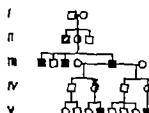
VI 8 (C.B.K.) Born 1913, died 1953 of gastrointestinal haemorrhage.

IV 36 (Sköld II 40) (T.A.) Born 1925 died 1950 of uraemia after a renal haemorrhage. He had repeated haemarthrosis and was disabled He attended a special school and was hospitalized several times at an orthopedic clinic for reconstructive treatment of knee and elbow joints. He had disablement pension and had no occupation

V 1 (Sköld I 1) (X.B.) Born 1930 died 1940 of postoperative haemorrhage after haematoma in the head

V 10 (Sköld I 7) (N.G.W.) Born 1930 First symptom of haemophilia at one year of age (gastrointestinal haemorrhage) Repeated haemarthroses, which have caused a stiff knee joint and impaired function of the elbow joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages, and have received at least 100 blood transfusions. He has attended a special school for disabled children. He lives on disablement pension and has no occupation

Family 19 Haemophilia A, clinically severe form.



III 1 (Sköld II 1) (X.B) Born 1891 died 1898 after a spinal disease. He always bled easily after tooth extractions and had gastrointestinal haemorrhages.

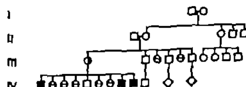
III 3 (Sköld II 3) (H.B) Born 1888 died 1907 after an appendectomy He had frequent haemorrhages from wounds and in muscles. Studied medicine. Case history published by Dahlgren in Hygiea in 1908

III 4 (Sköld II 4) (S.B) Born 1889 died 1953 of a cardiac infarction. He bled easily from wounds. Worked as a journalist.

V 4 (S.S) Born 1915. First symptom of haemophilia at three years of age (haemarthrosis) Late debute of the disease owing to poliomyelitis at six months of age He has had repeated joint bleedings, especially in ankle joint. He has not received blood transfusions. He can follow school education excellently but has been away from school for a whole term.

V 8 (M.X.) Born 1932. He has had haemarthrosis and is said to bleed easily

Family 20, Haemophilia, clinically severe form.

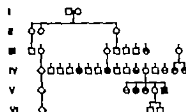


IV 1 (Sköld I 1) (K.D) Born 1892 died 1913 of gastrointestinal haemorrhages. Repeated haemarthrosis, gastrointestinal and renal haemorrhages. He had schizophrenia and died in a mental hospital.

IV 8 (Sköld I 8) (E.D) Born 1910 died 1912 of haemorrhage after a wound in the mouth.

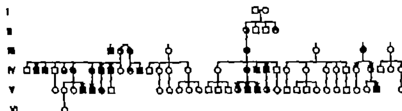
IV 9 (Sköld I 9) (V.D) Born 1913 died 1918 of intracranial haemorrhage Repeated haemarthrosis, gastrointestinal and renal haemorrhages. He had no occupation.

Family 26, Haemophilia, clinically severe form.



V 5 (Sköld III 12) (F.H.) Born 1889 died 1941 of intracranial haemorrhage. He had repeated haemarthrosis and was severely disabled, but he could work as a comptroller

Family 27, Haemophilia A, moderate form.



III 1 (Sköld III 1) (W.N.) Born 1882, died 1907 of wound haemorrhages after an appendectomy H always had a tendency to bleed.

IV 2 (Sköld II 2) (A.P.) Born 1907 First symptom of haemophilia at 5 years (haemarthrosis) Repeated haemarthrosis, whi h has impaired the function of the right shoulder nd ankle joint and the left knee joint. Hospitalized se eral times for joi t bleedings and has received about 20 blood and plasma transfusions. He could attend school fairly ormally and has been in ctive military ser vice. H is construction foreman in the concrete industry

IV 3 (Sköld II 3) (E.H.) Born 1910 First symptom of haemophilia at three years of age (subcutaneous haemorrhages) Repeated haemar throsis, whi h has impaired the function of the right knee and nkle joint. He cannot move the left leg due to an organized haematoma in the left side of the trunk. He could ttend school normally Free from military service. Hospitalized a few times for haemarthrosis and renal haemorrhages and has received about 10 blood transfusions. H works as a basket weaver

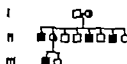
IV 8 (Sköld II 7) (M.H.) Born 1921 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) R peated haemarthroses, which have slighly impaired the function of the knee and ankle joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages, and has received about 300 blood and plasma transfusions and AHF preparations. He could attend school normally and is free from military service. He works as a tailor

IV 9 (Sköld II 8) (G.H.) Born 1922. First symptom of haemophilia at 7 years of age (excessive haemorrhage from a wound in the head) Repeated haemarthroses, which have moderately impaired the function of the ankle knee and elbow joints. He has been hospitalized a few times and received

VI 10 (E.K.) Born 1951 First symptom of haemophilia at *one year of age* (subcutaneous haemorrhages) Repeated haemarthroses, which has not disturbed the joint functions. Hospitalized a few times, but has not received any *blood transfusions*. He can attend ordinary school.

VII 2 (X.K.) Born 1959 died 1959 probably of haemophilia.

Family 23, Haemophilia, clinically severe form.



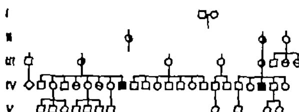
II 1 (Sköld II 1) (R.J.) Born 1905 died 1907 of haemophilia

II 5 (Sköld II 5) Born 1911 died 1911 *of haemophilia.*

II 7 (Sköld II 7) (H.J.) Born 1919 died 1922 of haemophilia (bleeding to death from external wound)

III 1 (Sköld I 1) (A.F.) Born 1931 died 1952 of intracranial haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages)

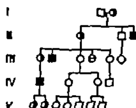
Family 24, Haemophilia, clinically severe form.



IV 8 (Sköld II 8 (K.G.) Born 1908, died 1951 of intracranial haemorrhage. *First symptom of haemophilia at* 6 month of age (subcutaneous haemorrhages) He had repeated haemarthrosis and was severely disabled. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages. Worked as a gardener

IV 20 (Sköld II 9) (K.K.) Born 1901 died 1916 of excessive haemorrhage

Family 25, Haemophilia, clinically severe form.



II 3 (Sköld III 3) (X.S.) At the age of 10 died of intracranial haemorrhage after a head injury

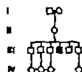
III 2 (Sköld II 2) (S.K.) Born 1888, died 1927 of septicaemia He had repeated haemarthrosis and a few gastrointestinal haemorrhages.

IV 1 *(Sköld I 1) (B.G.) Born 1914* died 1949 of interior bleeding after ileus. First symptom of haemophilia at 9 years of age (renal haemorrhage) He had repeated haematurias but few haemarthrosis. Hospitalized a few times for haematuria and a *fracture of left* tibia He worked as a shop attendant.

VI 1 (Sköld I 1) (S.W.) Born 1936. First symptom of haemophilia at tw months of age (subcutaneous haemorrhages). Repeated haemarthrosis, which have caused stiffness in both ankles joints and impaired function of the right knee. H has been hospitalized several times for haemarthrosis and gastrointestinal haemorrhages, and has received about 170 blood transfusions. He could attend school fairly normally and is free from military service. H works as a draughtsman.

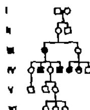
VI 5 (A.K.) Born 1959. First symptom of haemophilia at one year of age (subcutaneous haemorrhages).

Family 29, Haemophilia, clinically severe form.



III 4 (Sköld II 4) Born 1906, died 1937 of gastrointestinal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis. Worked as a travelling salesman.

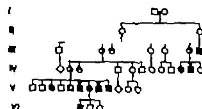
Family 30, Haemophilia, clinically severe form.



IV 2 (Sköld II 2) (B.K.) Born 1896, died 1900 of nasal haemorrhage.

IV 4 (Sköld II 4) (K.K.) Born 1908, died 1951 of renal haemorrhage. First symptom of haemophilia at one year of age (nasal haemorrhage). Repeated haemarthrosis, which caused a moderate disability. He had no occupation.

Family 31, Haemophilia B, severe form.



IV 10 (Sköld II 14) (K.O.K.) Born 1913. First symptom of haemophilia at birth when he bled from the umbilical cord. Repeated haemarthroses which have caused impaired function of the knee joints. H has been hospitalized several times for joint, renal and gastrointestinal haemorrhages and has received 10 blood transfusions. He could attend school fairly normally and is free from military service. H has worked as a farmer but is now disabled, and has disablement pension.

V 6 (Sköld I 6) (K.P.) Born 1932. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses in all the main joints, which have caused a stiff knee. Treated several times in hospital for haemarthrosis, gastrointestinal and renal haemorrhages. He could attend school normally and is free from military service. He is a factory worker.

about 50 blood transfusions. Could attend school normally and is free from military service He works as a goldsmith.

IV 12 (Sköld II 11) (O.A.) Born 1914 died 1930 of haemophilia.

IV 19 (Sköld II 13) (F.A.) Born 1916 died 1923 of haemophilia.

IV 20 (Sköld II 14) (G.A.) Born 1919 First symptom of haemophilia at 4 years of age (haemarthrosis) Repeated haemarthrosis, which caused impaired function of the knee and elbow joints. He has been hospitalized a few times for haemarthrosis, gastrointestinal and renal haemorrhages and has received about 25 blood transfusions. He could attend school normally and is free from military service. He works as a manufacturer of leather clothes.

V 4 (Sköld I 3) (O.P.) Born 1941 First symptom of haemophilia at one year of age (haemarthrosis) Repeated haemarthroses in ankle knee and elbow joints, which have moderately impaired the function of these joints. Hospitalized a few times and has received about 15 blood transfusions. He has been able to attend ordinary school and is free from military service. Works as an optician.

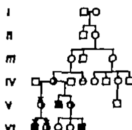
V 5 (Sköld I 4) (A.A.) Born 1941 First symptom of haemophilia at six months of age (excessive haemorrhages after an operation) Repeated haemarthroses which have caused moderately impaired function of the right knee and shoulder joint. He has been hospitalized a few times and has received about 10 blood transfusions. He could attend ordinary school and is free from military service Works as an optician

V 17 (L.J.) Born 1946 First symptom of haemophilia at 6 years of age (subcutaneous haemorrhages) Repeated haemarthrosis in the ankle knee and elbow joints, which has impaired the function of the right knee joint. He has received only one blood transfusion and has been hospitalized a few times. He can attend ordinary school but is free from gymnastics and handicraft.

V 18 (B.J.) Born 1947 First symptom of haemophilia at 6 years of age (subcutaneous haemorrhages) He has had a few haemarthroses but has normal function of the joints. He has been hospitalized a few times for an abdominal bleeding and tooth extraction and has received 12 blood transfusions. He can attend school normally

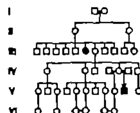
V 20 (B.K.) Born 195? First symptom of haemophilia at 9 months of age (haemorrhages from a wound in the mouth) Repeated haemarthroses in the ankle and knee joints, which have moderately impaired the function of the ankle joints. He can attend normal school and has not yet received any blood transfusions.

Family 28, Haemophilia A, severe form.



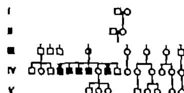
V 2 (Sköld II 2) (G.S.) Born 1930 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which caused impaired function of both knees. Later on the knee function has been without remarks. Hospitalized several times and have received 10 blood transfusions. He works as a journalist.

Family 34, Haemophilia A, severe form.



V 10 (Sköld II: 10) (O.L.) Born 1938. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which has caused a stiff right knee. Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 12 blood transfusions. He could attend school normally and is free from military service. He is a factory worker

Family 35, Haemophilia A, severe form.



IV 4 (Sköld I 4) (R.L.) Born 1917 First sympt m of haemophilia at one year of age (excessive haemorrhage from a wound) Repeated haemarthrosis, which has impaired the function of the knee and elbow joints. Hospitalized about 1—2 month a year for haemarthrosis, gastrointestinal and renal haemorrhages, nd have received about 700 blood and plasma transfusions nd AHF-preparations. He could not ttend school regulary and is free from military service. H works as onsultant gineer

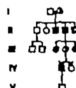
IV: 5 (Sköld I 7) (X.L.) Born 1920 died t a few weeks after birth of haemorrhages from the umbilicus.

IV 6 (Sköld I 5) (B.L.) Born 1922, died 1925 of haemorrhages from a wound.

IV 7 (Sköld IV 6) (K.L.) Born 1923, died 1926 f haemorrhages from a wound in the ha d.

IV 9 (Sköld I 9) (H.L.) Born 1926. First symptom of haemophilia at two years of age (subcutaneous haemorrhages) Repeated haemarthrosis, which has caused impaired functi n of both knee and elbow joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages. He has recei ed about 400 blood transfusions. H could not ttend school, but w s given private education in his home and was free from military service. He works as a bicycle repairman.

Family 36, Haemophilia A, severe form.



II 3 (Sköld III: 3) (X.D.) Born 1859, died 1871 of haemorrhages after tooth extraction.

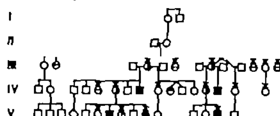
II 4 (Sköld III 4) (J.D.) Born 1861 died 1900 probably of intracranial nial haemorrhages. Severely disabled.

V 8 (Sköld I 8) (K.A.P.) Born 1930 died 1960 of haemorrhages in the mouth. First symptom of haemophilia at one year of age (subcutaneous haemorrhages.) Repeated haemarthrosis, which had caused severe disability. Treated several times in hospital and received about 25 blood transfusions. Could not attend school regulary, was free from military service. He had no occupation and was in a hospital for disabled.

V 9 (Sköld I 9) (B.P.) Born 1937 died 1942 of haemorrhages from the nose and mouth.

VI 1 (L.P.) Born 1943. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses, which have caused impaired function of the knees. Hospitalized a few times, once for a gastrointestinal haemorrhage. He has received 3 blood transfusions.

Family 32, Haemophilia A, severe form.



IV 8 (Sköld II 8) (R.K.) Born 1927 died 1957 of intracranial haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). He had repeated haemarthrosis and was hospitalized several times. He received about 40 blood transfusions. He was free from military service and worked as a draughtsman.

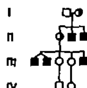
IV 14 (Sköld II 14) (P.A.) Born 1931 died 1959 of intracranial haemorrhage. First symptom of haemophilia at one year of age (nasal haemorrhages). He had repeated haemarthrosis, which caused impaired function of the ankle, knee and elbow joints. He could not attend school regulary, was free from military service and had no occupation.

V 6 (Y.T.) Born 1945 died 1945 soon after birth in haemorrhages.

V 9 (U.T.) Born 1957. She has female external genital characteristics, but shows a male sex-chromatin pattern. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). She has received about 10 blood transfusions.

V 13 (T.S.) Born 1953. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses in the knee and ankle joints which has not yet caused impaired function. He has received about 10 blood transfusions and a few times also AHF preparation. He can attend ordinary school.

Family 33, Haemophilia, clinically severe form.



II 2—3 (Sköld III 2—3) Died of excessive haemorrhages in youth.

III 1 (Sköld II 1) (K.K.) Born 190 died 1930 of intracranial haemorrhages.

III 2 (Sköld II 2) (X.K.) Born 1907 died 1911 of a bleeding from a wound in the lip.

III 5 (Sköld II 5) (E.K.) Born 1913 died 1913 of gastrointestinal haemorrhages. Repeated haemarthrosis which impaired the joint function. Worked as a gardener.

IV 5 (Sköld II 10) (Born 1890 died of umbilical haemorrhages about one month after birth.

IV 9 (*Sköld II 14*) (H.P.) Born 1898, died 1941 of gastrointestinal haemorrhages. First sympt m of haemophilia t one year of age (ex cessive haemorrhages from a wound) Repeated haemathrosis and gastrointestinal ha morrhages. Hospitalized 13 times fo gastrointesti al haemorrhages and recei ed about 15 blood transfusions. He worked s a glassblower

IV 12 (Sköld II 17) Died of umbilical haemorrhage shortly after *birth*.

IV 13 (Sköld II 18) Died of umbilical haem rrhages.

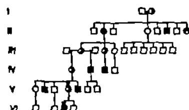
V 2 (Sköld I) (B.O.) Born 1928, died 1947 of intracranial *haemorrhage*

First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemar throsis. Hospitalized several times *for* haemarthrosis, gastrointesti nal and renal haemorrhages and received about 80 blood transfu si ns.

V 5 (Sköld I 4) (S.A.W.) Born 1930 died 1939 of intracranial haemorrhage. First symptom of haemophilia at one year of age (subcutaneous *haemorrhages*) Repeated haemarthrosis, especially in th knee joints, and gastrointestinal haemorhages. Hospitalized several times and received about 10 blood transfusi ns.

V 10 (Sköld I 9) (S.H.) *Born* 1931, died 1932 of i tracranial haemorrhge.

Family 40, Haemophilia A, severe form.



II 6 (Sköld V 6) (P.E.) Born 1860, died 1862 of haemorrh ges.

IV 2 (Sköld III 2) (N.O.) *Born* 1907 d ed 1947 of uraemia fter renal haemorrhage First symptom of haemophilia t on year of age (subcutaneous haem rrhages) Repeated haemarthrosis, whi h sed impaired function of the right elbow He was treated se eral times in hospital for haematuria nd received few blood transfusions. H worked as a gineer d was free from military service

IV 3 (Sköld III 3) (O.O.) Born 1906, died 1937 f uraemia after renal haem rrhage First symptom f haemophilia at one year of age (subcutaneous haemorrhages) Re peated haemarthrosis, which caused impaired mobility in th *knee and lbow joints. He* worked as a sculptor

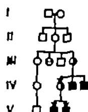
V 2 (Sköld II 2) (L.P.) Born 1924 died 1942 of gastrointestinal haemorrhage *First symptom of haemophilia* t one year of age (subcutaneous haemorrhages) H had repeated haemarthrosis, which impaired the function of the knee and lbow joints. He received bout 10 blood transfusions.

V 5 (Sköld II 5) (K.P.) Born 1932, died 1933 of haemorrhages from wou d in the tongue

VI 3 (S.H.) Born 1952. First ymptom of haemophilia t on year of age (subcutaneous haemorrhages) Repeated haemarthroses, which have not y t impaired the joint func tion. Hospitalized few times and has received about 10 blood- and plasma trans*fusions*. H can ttend ordinary school.

IV 1 (Sköld I 1) (L.L.) Born 1929 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which has caused a practically stiff left knee and a right knee with impaired function The ankle joints are moderately impaired Hospitalized several times for joint bleedings and has received blood transfusions regulary once a month during the years 1941—1956. He received about 300 blood and plasma transfusions. He could attend school normally and works as an engineer

Family 37 Haemophilia A, severe form.

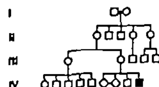


IV 3 (Sköld I 2) (T.L.) Born 1930 died 1951 of haemorrhages after a traffic accident First symptom of haemophilia at $1^1/_2$ years of age (excessive haemorrhages from a wound in the lip)

V 2 (C.S.) Born 1951 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) Repeated haemarthroses in all the main joints which have caused a practically stiff left knee Hospitalized several times and have received about 50 blood transfusions and AHF preparations. He can attend school fairly normal

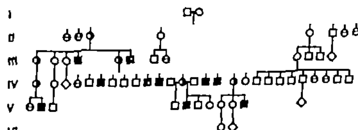
V 3 (L.C.S.) Born 1958. First symptom of haemorphilia at four month of age (subcutaneous haemorrhages) Repeated haemarthrosis.

Family 38, Haemophilia, clinically severe form.



IV 7 (Sköld I 4) (E.N.) Born 1900 died 1941 of renal haemorrhage First symptom of haemophilia at one year of age (subcutaneous haemorrhages) He had repeated haemarthrosis, which caused him difficulty in walking. Hospitalized several times for gastrointestinal and renal haemorrhage He had no occupation.

Family 39 Haemophilia, clinically severe form.



III 4 (Sköld III 4) (E.M.) Born 1865 died of haemophilia in youth.

III 6 (Sköld III 6) (A.M.) Born 1874 died of haemophilia at an early age

IV (Sköld II 10) (Born 1890 died of umbilical haemorrhages about one month after birth.

IV 9 (Sköld II 14) (H.P.) Born 1898, died 1941 of gastrointestinal haemorrhages. First symptom of haemophilia at one year of age (excessive haemorrhages from a wound). Repeated haemathrosis and gastrointestinal haemorrhages. Hospitalized 13 times for gastrointestinal haemorrhages and received about 15 blood transfusions. He worked as a glassblower.

IV 12 (Sköld II 17) Died of umbilical haemorrhage shortly after birth.

IV 13 (Sköld II 18) Died of umbilical haemorrhages.

V 2 (Sköld I 2) (B.O.) Born 1928 died 1947 of intracranial haemorrhage. First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages). Repeated haemarthrosis. Hospitalized several times for haemarthroses, gastrointestinal and renal haemorrhages and received about 30 blood transfusions.

V 5 (Sköld I 4) (S.A.W.) Born 1930 died 1939 of intracranial haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis, especially in the knee joints, and gastrointestinal haemorhages. Hospitalized several times and received about 10 blood transfusions.

V 10 (Sköld I 9) (S.H.) Born 1931 died 1932 of intracranial haemorrhage.

Family 40, Haemophilia A, severe form.

FAMILY 40

I

II

III

IV

V

VI

II 6 (Sköld V 6) (P.E.) Born 1860 died 1862 of haemorrhages.

IV 2 (Sköld III 2) (N.O.) Born 1907 died 1947 of uraemia after renal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis, which caused impaired function of the right elbow. He was treated several times in hospital for haematuria and received a few blood transfusions. He worked as an engineer and was free from military service.

IV 3 (Sköld III 3) (O.O.) Born 1906, died 1937 of uraemia after renal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis, which caused impaired mobility in the knee and elbow joints. He worked as a sculptor.

V 2 (Sköld II 2) (L.P.) Born 1921 died 1942 of gastrointestinal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). He had repeated haemarthrosis, which impaired the function of the knee and elbow joints. He received about 10 blood transfusions.

V 5 (Sköld II 5) (K.P.) Born 1932, died 1933 of haemorrhages from a wound in the tongue.

VI 3 (S.H.) Born 1952. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses, which have not yet impaired the joint function. Hospitalized a few times and has received about 10 blood and plasma transfusions. He can attend ordinary school.

IV 1 (Sköld I 1) (L.L.) Born 1929 First symptom of haemophilia at *one year of age* (subcutaneous haemorrhages) Repeated haemarthrosis, which has caused a practically *stiff left knee and a right* knee with impaired function The ankle joints are moderately impaired Hospitalized several times for joint bleedings and has received blood transfusions regulary once a month during the years 1944—1956 He received about 300 blood and plasma transfusions. *He* could attend school normally and works as an engineer

Family 37 Haemophilia A, severe form.

FAMILY 37

IV 3 (Sköld I 2) (T L.) Born 1930 died 1954 of haemorrhages after a traffic accident. First symptom of haemophilia at $1^1/_2$ years of age (excessive haemorrhages from a wound in the lip)

V 2 (C.S) Born 1951 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) Repeated haemarthroses in all the main joints which have caused a practically stiff left knee. Hospitalized several times and have received about *50 blood* transfusions and AHF-preparations. He can attend school fairly normal.

V 3 (L.C.S.) Born 1958. First symptom of haemorphilia at four month of age (subcutaneous haemorrhages) Repeated haemarthrosis.

Family 38, Haemophilia, clinically severe form.

FAMILY 38

IV 7 (Sköld I 4) (E.N) Born 1909 died 1941 of renal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) He had repeated haemarthrosis, which caused him difficulty in walking Hospitalized several times for gastrointestinal and renal haemorrhage. *He had* no occupation

Family 39, Haemophilia, clinically severe form.

FAMILY 39

III 4 (Sköld *III* 4) (E.M.) Born 1865 died of haemophilia in youth

III 6 (Sköld III 6) (A.M.) Born 1874 died of haemophilia at an early age.

IV (Sköld II 10) (Born 1890, died of umbilical haemorrhages about one month after birth.

IV 9 (Sköld II 14) (H.P.) Born 1898, died 1941 of gastrointestinal haemorrhages. First symptom of haemophilia at one year of age (excessive haemorrhages from a wound) Repeated haemathrosis and gastrointestinal haemorrhages. Hospitalized 13 times for gastrointestinal haemorrhages and received about 15 blood transfusions. He worked as a glass-blower

IV 12 (Sköld II 17) Died of umbilical haemorrhage shortly after birth.

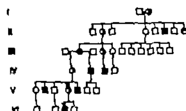
IV 13 (Sköld II 18) Died of umbilical haemorrhages.

V 2 (Sköld I 2) (B.O.) Born 1928, died 1947 of intracranial haemorrhage First symptom of haemophilia at 6 months of age (subcutaneous haemorrhages) Repeated haemarthrosis. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages and received about 40 blood transfusions.

V 5 (Sköld I 4) (S.A.W.) Born 1930, died 1939 of intracranial haemorrhage First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, especially in the knee joints, and gastrointestinal haemorhages. Hospitalized several times and received about 10 blood transfusions.

V 10 (Sköld I 9) (S.H.) Born 1931 died 1932 of intracranial haemorrhage.

Family 40, Haemophilia A, severe form.



II 6 (Sköld V 6) (P.E.) Born 1860 died 1882 of haemorrhages.

IV 2 (Sköld III 2) (N.O.) Born 1907 died 1947 of uraemia after renal haemorrhage First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which caused impaired function of the right elbow He was treated several times in hospital for haematuria and received a few blood transfusions. He worked as an engineer and was free from military service

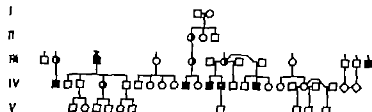
IV 3 (Sköld III 3) (O.O.) Born 1906, died 1937 of uraemia after renal haemorrhage First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which caused impaired mobility in the knee and elbow joints. He worked as a sculptor

V 2 (Sköld II 2) (L.P.) Born 1924 died 1942 of gastrointestinal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) He had repeated haemarthrosis, which impaired the function of the knee and elbow joints. He received about 10 blood transfusions.

V 5 (Sköld II 5) (K.P.) Born 1932, died 1933 of haemorrhages from a wound in the tongue

VI 3 (S.H.) Born 1952. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses, which have not yet impaired the joint function. Hospitalized a few times and has received about 10 blood- and plasma transfusions. He can attend ordinary school.

Family 41, Haemophilia B, mild form.



III 3 (Sköld II 3) (J.E.O.) Born 1885. First symptom om haemophilia at 6 months of age (excessive haemorrhages from a wound in the mouth). No haemarthrosis, but a few gastrointestinal and repeated renal haemorrhages. Treated several times in hospital and have received about 25 blood transfusions. He could attend school normally and was in military service about 5 months. He now lives on ordinary pension and worked as a lift repairman.

III 10 (Sköld III 14) (K.R.O.) Born 1904. First symptom of haemophilia at 7 years of age (excessive haemorrhages from a wound in the head). A few times haemarthrosis after trauma. He could attend school normally and was free from military service. He works as a taxi driver and owns his taxi.

IV 1 (Sköld II 1) (S.K.) Born 1910, died 1939 of intraabdominal haemorrhages after a rupture of the spleen. He had repeated haematomata in the muscles and was hospitalized a few times and received a few blood transfusions. He worked as a gardener.

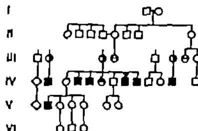
IV 10 (Sköld II 6) (E.O.) Born 1903, died 1930 of haemorrhages after appendectomy. He had always tendency to bleed.

IV 12 (Sköld II 7) (K.A.O.) Born 1914, died 1920 of haemorrhage after tonsillectomy.

IV 13 (Sköld II 8) (K.G.O.) Born 1916. First symptom of haemophilia at one year of age (excessive haemorrhages from a wound). Repeated haemarthrosis and gastrointestinal haemorrhage. He works as a lumberman.

IV 16 (K.L.) Born 1925. He has a tendency to bleed after tooth extraction and wounds. No haemarthrosis.

Family 42, Haemophilia A, severe form.



IV 1 (Sköld II 1) (K.G.P.) Born 1909, died 1940 of gastrointestinal haemorrhages. Repeated haemarthrosis, which impaired the knee function. He was hospitalized several times for renal and gastrointestinal haemorrhages. During his last years he was at a home for epileptics, he had epileptic fits possibly after a cerebral haemorrhage at twelve years of age.

IV 3 (Sköld II 3) (G.K.) Born 1910, died 1910 of excessive haemorrhages after an incision in a haematoma. He had repeated haemorrhages in the joints.

IV 4 (Sköld II 4) (E.K.) Born 1912, died 1933 of gastrointestinal haemorrhages. Repeated haemarthrosis, gastrointestinal and renal haemorrhages.

IV 7 (Sköld II 7) (B.R.) Born 1924 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthrosis, which has caused impaired function of the knee and elbow joints. Treated a few times in hospital and have received about 15 blood transfusions. He attended a special school for disabled children. He has no occupation

IV 8 (Sköld II 8) (B.K.) Born 1930 died 1936 of violent interior haemorrhage He had repeated haemarthrosis.

IV 11 (Sköld II 11) (K.N.) Born 1920 died 1940 of haemorrhage from perforating haemarthrosis in the left knee He had repeated haemarthrosis which caused impaired function of the knee joints.

V 1 (Sköld I 1) Born 1929 died 1936 of gastrointestinal haemorrhage He had repeated haemarthrosis.

Family 43, Haemophilia A, moderate form.

FAMILY 43

I

II

III

IV

III 7 (Sköld II 7) (S.P.) Born 1901 First symptom of haemophilia at 14 years of age (excessive haemorrhage after tooth extraction) He has had about 10 haemarthrosis in the knee joints. Joint function is good Hospitalized a few times for renal haemorrhage and has received about 10 blood transfusions. He could attend school normally and is free from military service. He works as a clerk.

III 8 (Sköld II 8) (K.P.) Born 1901 died 1933 in U.S.A. of haemorrhage after operation for nephrolithiasis.

III 9 (Sköld II 9) (B.P.) Born 1904 First symptom of haemophilia at 15 years of age (haemarthrosis) A few haemarthrosis in the knee and ankle joints. Good joint function. Once a renal haemorrhage. He has never received blood transfusions. Could attend school normally and is free from military service. He works as a clerk.

III 10 (Sköld II 10) (O.P.) Born 1906. First symptom of haemophilia at 6 years of age (excessive haemorrhages after tooth extraction) He has had about 5 haemarthrosis in the knee joints without causing impaired joint function. He has been hospitalized a few times for renal haemorrhage and has received about 15 blood transfusions. He could attend school normally and is free from military service He earns his living as a manufacturer of candles.

Family 44. Haemophilia B, severe form.

FAMILY 44

I

II

III

IV

V

VI

VII

VIII

IV 5 (A.C.) Born 1855 died 1858 of unknown bleeding.

V 2 (Sköld III 32) (A K.) Born 1875 died 1870 He had a tendency to bleed.

V 4 (Sköld III 33) (H K.) Born 1877 died 1880 of intracranial haemorrhage.

V 10 (Sköld III 1) (H G) Born 1888, died 1889 He always bled easily

V 11 (Sköld III 2) Born 1890 died 1949 of intracranial haemorrhages.

VI 1 (Sköld II 7) (H.B) Born 1903 died 1905 of intracranial haemorrhages.

VI 4 (Sköld II 10) (T.P) Born 1910 died 1949 of intracranial haemorrhage He had repeated haemarthrosis, which impaired the function of the knee and elbow joints. He worked as a tailor

VI 5 (Sköld II 11) (O.P) Born 1914 died 1925 of wound haemorrhages after having cut himself in the mouth with a nut shell.

VI 9 (J K.) Born 1916 died the same year of intracranial haemorrhage.

VI 10 (S.H.) Born 1925 died 1938 of intracranial haemorrhage. He had repeated haemarthrosis.

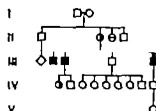
VI 17 (B.H) Born 1927 died 1930 of intracranial haemorrhage

VII 15 (R.F) Born 1944 died 1947 of intracranial haemorrhage

VII 17 (D.F) Born 1947 died 1948 of intracranial haemorrhage.

VII 24 (J O) Born 1955. First symptom of haemophilia at 5 months of age (subcutaneous haemorrhages) Repeated haemarthroses, especially in the ankle joints which have caused impaired function of these joints. Repeated hospitalization due to the joint bleedings. He has received about 10 blood transfusions.

Family 45, Haemophilia A, moderate form.

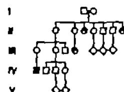


III 1 (G.S.) Born 1898. First symtom of haemophilia at 10 years of age (excessive haemorrhage from a wound) After traumata haemarthrosis in the knee joints. Hospitalized once for nephrolithiasis. He has never received any blood transfusions. Could attend school normally and was in military service. He was a printers assistant but since 1939 he has multiple sclerosis and is completely disabled

III 2 (M.S) Born 1900 died 1955 of intracranial haemorrhage after an accident. First symptom of haemophilia at four years of age (subcutaneous haemorrhages. Hospitalized a few times for gastrointestinal and once for celebral haemorrhage. A few times haemarthrosis in the knees but no impaired joint function He worked as a journalist.

III 4 (K.S.) Born 1904 died 1960 of uraemia after an operation for nephrolithiasis. First symptom of haemophilia at 15 years of age (excessive haemorrhage after a wound) A few haemarthrosis after light traumata in the knees, He had previously 1959 been operated for nephrolithiasis with nephrectomy under protection of AHF preparation and blood transfusions. Afterwards he got hepatitis, and recovered completely He worked as a printers assistent.

Family 46, Haemophilia A, severe form.



IV 1 (Sköld II 1) (B.S.) Born 1921 First symptom of ha mophilia at six months of age (nasal haemorrhage) Repeated haema throsis in the knee elbow and ankle joints, which has caused impaired function f the left ankle and both knees joints. He has been hospitalized several times for haemarthrosis and renal haemorrhages and has received about 50 blood transfusions. Could attend school fairly nor mally and is free from military service. He worked as a clerk, but has now disablement pension.

Family 47 Haemophilia, clinically severe form.



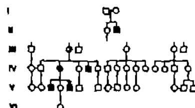
II 1 (Sköld III 1) (O.A.) Born 1807 died 1903 of haemophilia. He had repeated haemarthrosis and had one stiff knee.

II 5 (Sköld III 5) (O.X.A.) Born 1881 died 1911 of haemophilia.

II 6 (Sköld III 6) (F.A.) Born 1885, died 1911 of gastrointestinal haemorrhages.

IV 1 (Sköld I 1) (S.A.T.) Born 1931 died 1937 of cerebral haemorrhage. First symptom of haemophilia at 10 month of age (subcutaneous haemorrhages) He had repeated joint nd nasal haemorrhages.

Family 48, Haemophilia A, severe form.



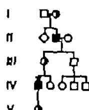
II 2 (Sköld IV 2) Suspected t be haemophili died t 40 years of age He had frequent nasal and joint haemorrhages.

IV 4 (Sköld II 7) (T.S.) Born 1905, died 1918 of excessi haemorrhage fter puncture of on knee. He had frequent nasal and joint haemorrhages.

V 1 (Sköld I: 10) (T.W.) Born 1920, died 1927 of nasal haemorrhage. He had repeated haemarthrosis and nasal haemorrhage but was never hospitalized

V 3 (Sköld I 12) (L.W.) Born 1926. First sympt m of haemophilia at on year of age (haemarthrosis) Repeated haemarthrosis, which has caused severe impairment of the function in the knee nd elbow joints. He must have bandages for beeing able to work. Hosp talized several times for ha marthrosis, gastrointestinal and renal haemorrhages nd has received about 150 blood transfusions. He attended privat teaching in the home and is free from military service.

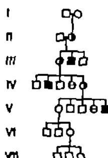
Family 49 Haemophilia B, moderat form.



II 1 (A.E.) Born 1867 died 1809 of intracranial haemorrhage. He always bled easily

IV 1 (Sköld I 1) (S.Ö.) Born 1921 First symptom of haemophilia at six months of age (excessive haemorrhage from wound in the mouth) Repeated *haemarthrosis, which has not caused any impaired joint function. Treated in* hospital a few times and has received *about 50 blood transfusions.* He has had one gastrointestinal and one renal haemorrhage He could attend school normally and is free from military service. He works as a printers assistant.

Family 50, Haemophilia, clinically severe form.

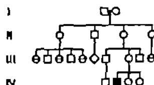


III 2 (Sköld V 2) (X.E.) Died of haemorrhage after a bit in the tongue in an early age.

IV 2 (Sköld IV 2) (X.B.) Died of haemophilia in an early age

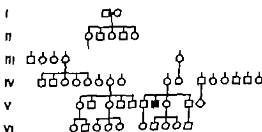
V 5 (Sköld III 5) (E.H.) Born 1895, died 1919 of pneumonia in hospital after a haemorrhage in the bottom of the mouth. Repeated haemarthrosis, which caused impaired function of the knee and ankle joints. *He had repeated renal* haemorrhages. He worked as *a journalist.*

Family 51, Haemophilia, clinically severe form.



IV 2 *(Sköld I 3) (B.A.)* Born 1938, died 1949 of haemorrhages after *appendectomy He had* hydrocephalus and repeated haemorrhages *in the joints and from* wounds.

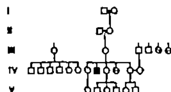
Family 52, Haemophilia, clinically severe form.



V 7 (Sköld II 7) (S.J.) Born 1914, died 1947 after suicidium. First symptom of haemophilia at four years of age (subcutaneous haemorrhage) Repeated haemarthrosis which did not impaire the joint function. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages,

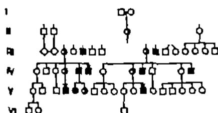
and received about 100 blood transfusions. He was Rh-n gall e nd was immunized with anti D and had once a severe haemolytic transfusion reaction with anuria. H worked as a photographer and was in active military service *during the second world war*

Family 53, Haemophilia, clinically severe form.



IV 8 (Sköld II:1) (F.N.) Born 1899 died 1930 of intracranial haemorrhage. First ymptom of haemophilia at six months of age (subcutaneous haemorrhages) Repeated haemarthrosis, which caused imp ired function of all the main joints. Hospitalized se eral times for haemarthrosis and renal haemorrhages. He worked as a business man.

Family 54, Haemophilia A, mild form.



III 3 *(Sköld III 7) (K.L.) Born 1884* died 1915 of cancer recti. H always had strong t nd ney to bleed from the nose and after tooth extractions. He had haemarthrosi in the knee joi ts, which caused moderately impaired functi n of th knees.

III 7 (Sköld III 11) (H.I.) Born 1893, died 1915 of xcessive haemorrhges fter n ccident. He had repeated subcutaneous nd nasal haemorrhages d few haem throsis.

IV 5 (Sköld II 5) (K.E.K.L) Born 1918, died 1931 of xcess haemorrhge after ppendectomy First ymptom of haemophilia at two years of ge (subcutaneous haemorrhages) H had repeated haemarthrosis, especially in the knee joints, but no impaired joint function. R peated gastrointestinal haemorrhages.

IV 6 (Sköld II 6) (E.K.) Born 1919 First symptom f haemophilia at three years of age (nasal haemorrhages) Repeated nasal haemorrhages nd haemarthrosis, especially in the knee joints, but n impaired j int function. Hospitalized few times for haemorrhages after tooth extractions and renal haemorrhages, has received about 50 blood a d plasma transfusions. He could tt nd school normally and is free from *military service. H* works as a clerk.

IV 9 (Sköld II 9) (F.N.) Born 1919 died 1921 of excessive haemorrhage from a wound in the mouth.

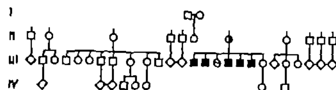
IV 12 (Sköld II 12) (B.N.) Born 1922. First symptom of haemophilia t si months of age (subcutaneous haemorrhages) Repeated subcutaneous and excessive haemorrhages after tooth extractions. No haemarthrosis. He had attended school *normally*

V 4 (Sköld I 3) (K.E.K.) Born 1937 First ymptom of haemophilia t one year of *age (asal haemorrhage)* Repeated nasal and subcutaneous haemorrhages. *No haemar*

throsis. Has received about 20 transfusions. Is free from military service. He has attended school normally and works as a clerk.

V 12 (L.G.N.) Born 1949. First symptom of haemophilia at two years of age (excessive haemorrhage from a wound in the mouth). Repeated haemarthrosis, but no impaired joint function. Hospitalized once for a bleeding in the mouth. No transfusions. He can attend school fairly normally, is free from gymnastics and handicraft.

Family 55, Haemophilia clinically severe form.



III 11 (Sköld II 35) (E.E.) Born 1904, died 1935 of intracranial haemorrhage. Repeated haemarthrosis.

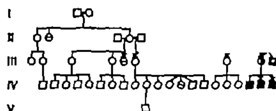
III 12 (Sköld II 36) (H.E.) Born 1907, died 1917 o excessive haemorrhage from a wound in the mouth. Repeated haemarthrosis.

III 14 (Sköld II 38) (S.E.) Born 1913, died probably of an intracranial haemorrhage at one month of age.

III 15 (Sköld II 39) (T.E.) Born 1915, died 1916 of haemorrhage from a wound in the mouth.

III 16 (Sköld II 40) (G.E.) Born 1916, died 1920 of bleeding from a wound in the thumb.

Family 56, Haemophilia B, severe form.

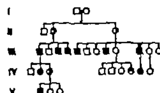


IV 19 (Sköld II 19) (B.J.) Born 1920, died 1943 of intracranial haemorrhage. First symptom of haemophilia at one year of age (nasal haemorrhage). Frequent nasal and joint bleedings which caused stiff knee. Hospitalized several times for gastrointestinal and renal haemorrhage. He attended a special school for handicapped children. No occupation, lived on disability pension.

IV 20 (Sköld II 20) (A.J.) Born 1925. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis, which have caused a stiff right knee and impaired function of the left elbow joint. Hospitalized a few times for gastrointestinal and renal haemorrhage and has received about 100 blood transfusions. He attended a special school for handicapped children, is free from military service and works as a book binder.

IV 21 (Sköld II 21) (G.J.) Born 1931. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthrosis, which have caused impaired function of the main joints. Hospitalized for intracranial haemorrhage at two years of age and several times for haemarthrosis and has received about 100 blood transfusions. He could attend school fairly normal and is free from military service. He has no occupation and lives on disability pension.

Family 57 Haemophilia A, severe form.



III 1 (Sköld II 1) (K.S.) Born 1886 died 1952 of haemophilia in his home, probably f gastrointestinal haemorrhages. Repeated haemarthrosis, which caused impaired function of both knee joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages, and received about 20 blood transfusions. He worked as a precision mechanic.

III 3 (Sköld II 2) (H.S.) Born 1890 died of haemophilia in an early ge. H had repeated haemarthrosis.

III 4 (A.S.) Born 1892, died 1903 of gastrointestinal haemorrhage.

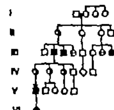
III 5 (Sköld II 4) (O.S.) Born 1890, d ed 1899 of haemophilia. He always had tendency to bleed

III 8 (Sköld II 7) (R.S.) Born 1900 died 1927 of pulmonary haemorrhage. Repeated haemarthrosis, which caused severe disability H had T.B. and was hospitalized several times for gastrointestinal and pulmonary haemorrhages. H had no occupation

III 10 (Sköld II 9) (J.S.) Born 1906. First symptom f haemophilia at one year of age Repeated haemarthroses whi h have caused impaired function of the knee and nkle j ints. Hospitalized several times for joint and renal haemorrhages, and has received about 10 blood transfusions. H could attend school normally and is free from military service He works as a gardener

V 1 (H.B.) Born 1947 First symptom of haemophilia at two years of age (subcutaneous haemorrhages) Repeated haemarthrosis in the knee and ankle joints, which have caused impaired function of the left knee joint. Hospitalized a few times for haemarthrosis and gastrointestinal haemorrhage, and has received about 20 blood transfusions. He can attend school fairly normally

Family 58, Haemophilia A, moderate form.

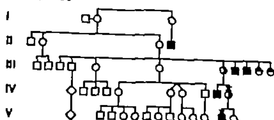


III 2 (Sköld III 2) (R.J.) Born 1883, died 1934 of renal haemorrhage. He had repeated haemarthrosis but no impaired joint function. Hospitalized a few times for gastrointestinal and renal haemorrhage He worked as a baker

III 3 (Sköld II 3) (T.J.) Born 1885, died 1914 of interior haemorrhage after an accident. He had repeated gastrointestinal haemorrhages and haemarthrosis.

V 1 (Sköld I: 1) (U.H.) Born 1932. First symptom of haem philia t two years of age (subcutaneous hae morrhage) No haemarthrosis. Hospitalized a few times for haemorrhage after tooth extractions, renal haemorrhage and peritonitis, and has received about 5 blood transfusions. He could attend school normally and is free from military service. He works as a baker

Family 59 Haemophilia B, severe form.



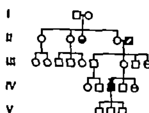
III 8 (Sköld II 1) (V.S.) Born 1891 died 1895 of haemorrhage from a wound in the mouth. He always bled easily

III 9 (Sköld II 5) (A.S.) Born 1894 died 1945 of uraemia after renal haemorrhage Repeated haemarthrosis, which caused a stiff right knee. Hospitalized several times for haemarthrosis and renal haemorrhage. He worked as a farmer

IV 8 (P.M.) Born 1924 died 1929 of excessive haemorrhage after an accident. He always bled easily

V 11 (R.B.) Born 1954 First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the mouth) He has had haemarthroses in the knee, ankle and elbow joints, which have caused impaired function of the right knee joint. Hospitalized for haemarthrosis and has received about 10 blood transfusions.

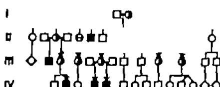
Family 60, Haemophilia B, severe form.



II 5 (X.J.) He is said to have a faulty joint and limped May have been a bleeder

IV 3 (Sköld II 3) (E.R.) Born 1892. First symptom of haemophilia at one year of age (excessive haemorrhage from a wound in the lip) Repeated haemarthroses which have caused severe disability Hospitalized several times for haemarthrosis, gastrointestinal and intramuscular haemorrhages, and has received about 400 blood and plasma transfusions. He attended school normally and was free from military service He worked as a tax inspector but is now retired and lives on pension.

Family 61, Haemophilia B, severe form.



II 4 (E.A.) Born 1900 died 1901 of haemophilia.

III 1 (W.A.) Born 1913 died 1937 of haemorrhage in the bottom of the mouth He had repeated haemarthrosis and the left knee was stiff He had no occupation

IV 2 (P.O.C.) Born 1941 First symptom of haemophilia at 6 months of age. Repeated haemarthrosis especially in the knee and ankle joints, which has caused a light impaired function of these joints. Hospitalized several times for haemarthrosis and renal haemorrhage and has received about 25 blood transfusions. He has attended ordinary school, but has been away from school about half the time He is now studying at a business school.

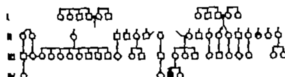
IV 4 (S.L.) Born 1940 First symptom of haemophilia at one year of age.

Repeated haemarthrosis, which has not yet caused any impaired joint function. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages and has received about 50 blood transfusions. He could attend ordinary school, but was away about one year. He is free from military service. H works as a clerk.

IV 5 (G.E.) Born 1944. First symptom of haemophilia at one year of age. Repeated haemarthroses, which have caused moderately impaired function of the left knee and wrist joint. Hospitalized several times for haemarthrosis and renal haemorrhages nd has received about 15 blood transfusions. H could attend ordinary school fairly normally. He is a factory worker.

Family 62, Haemophilia A, severe form.

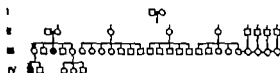
FAMILY 62



IV 2 (B.W.) Born 1942. First symptom of haemophilia at one y ar of age. Repeated haemarthrosis in ll the main joints, which has caused impaired function f th ankl knee and elbow joi ts. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages, and has received about 60 blood transfusions and AHF-preparations. He can attend rd nary school but is away for long periods.

Family 63, Haemophilia A, severe form.

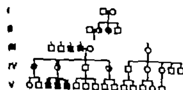
FAMILY 63



IV 1 (A.A.) Born 1938, died 1957 f haemorrhage after an bdominal peratio (ilius) F rst ymptom of haemophilia t on year f ge (subcutaneous haemorrhage). Repeated haemarthrosis, which caused impaired function of the mal joints. Hospitalized a few times, once for renal haemorrhage. He had received about 10 blood transfusions.

Family 64, Haemophilia B, mild form.

FAMILY 64



III 3 (C.S.) Born 1893, died 1947. Hospitalized several times for excessi haemorrhage after tooth extractions and operations. H had no haemarthrosis.

III:4 (V.S.) Born 1896, died 1917 of intracranial haemorrhage. Hospitalized for e cessive haemorrhage

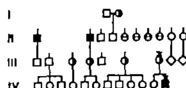
after tooth extractions. No haemarthrosis.

V 3 (J.F.) Born 1951. First symptom of haemophilia at five years of age (excessive haemorrhage after abrasio). He has also bled abnormally after tooth extraction. No haemarthrosis. Hospitalized two times and has received three blood transfusions. He attends school normally.

V 4 (P.F.) Born 1953. He has not yet had any bleeding episode. Attends school normally.

V 5 (N.F.) Born 1953. He has also not had any bleeding episode. Attends school normally.

Family 65, Haemophilia A, mild form.

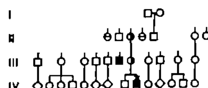


II 1 (V.W.) Born 1887. He has had excessive haemorrhage after tooth extraction and from wounds. No haemarthrosis. Worked as a telegraph worker.

II 2 (A.W.) Born 1889. He has bled abnormally after tooth extraction. No haemarthrosis.

IV 12 (B.J.) Born 1948. First symptom of haemophilia at five years of age (tooth extraction). Repeated abnormal bleedings after tooth extractions. No haemarthrosis. He can attend school normally.

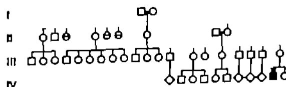
Family 66, Haemophilia A, severe form.



III 6 (H.N.) Born 1920, died 1942 of haemophilia.

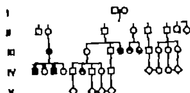
IV 6 (L.C.) Born 1955. First symptom at one year of age. A few haemarthrosis, which have not yet caused any impaired joint function. Hospitalized once and has received about 10 blood transfusions.

Family 67, Haemophilia A, severe form.



IV 6 (J.A.J.) Born 1947. First symptom of haemophilia at one year of age. Repeated haemarthrosis in all the main joints, which have caused impaired function of the knee, ankle and elbow joints. Walks with a rod. Hospitalized several times for haemarthrosis and has received about 150 blood transfusions. He cannot attend school, but receives private education at home two times a week.

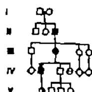
Family 68, Haemophilia A, severe form.



IV 1 (G.F.) Born 1930. First symptom of haemophilia at one year of age. Repeated haemarthroses in all the main joints, which have caused a stiff right knee and impaired function of the ther knee and elbow joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhage and has received about 60 blood transfusions. He could not finish school, but has later completed his examinations privately. He is free from military service, and works as a clerk.

IV 3 (O.F.) Born 1936. First symptom of haemophilia at one year of age. Repeated haemarthrosis, which ha e n t yet caused impaired function of the joints. Hospitalized a few times for renal haemorrh-ge and has received about 10 blood transfusions. He could at tend school normally. Is free from military service and works as a clerk.

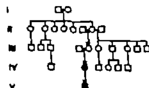
Family 69, Haemophilia B, mild form.



II 3 (S.S.) Born 1836, died 1908. He bled excessively after tooth extractions.

IV 1 (E.G.) Born 1910. First symptom of haemophilia at 30 years of age (tooth extraction). No other bleeding episodes. Attended school normally and has been in military service. He works as a dealer.

Family 70, Haemophilia A, severe form.



V 1 (S.M.) Born 1950. First symptom of haemophilia at two years of age (haemarthrosis). Repeated haemarthroses, which have caused slightly impai ed function of the knee and ankle joints. Hospitalized several times for haemarthrosis and bleeding after tooth extraction and has received about 40 blood transfusions. He can at tend school normally but is free from gymnastics.

Family 71 Haemophilia B, mild form.

FAMILY 71

III 6 (A.A.) Born 1880 died 1945 of gastrointestinal haemorrhage. He had always a bleeding tendency after operations. No haemarthrosis. He worked as a painter

III 7 (J.A.) Born 1884 First symptom of haemophilia at 10 years of age (postoperative haemorrhage after tonsillectomy) He has had postoperative haemorrhage after various operations and has been hospitalized several times. No haemarthrosis. He works as a carpenter

III 9 (A.G.) Born 1888 died 1958 First symptom of haemophilia at 7 years of age (postoperative haemorrhage) Repeated excessive haemorrhage after tooth extractions. No haemarthrosis.

V 1 (B.A.) Born 1941 First symptom of haemophilia at 7 years of age (bleeding after tooth extraction) Repeated excessive haemorrhage after tooth extractions and minor operations, and has received 12 blood transfusions. No haemarthrosis. He could attend school normally and is free from military service

V 2 (T.A.) Born 1944 died 1960 of intracranial haemorrhages. First symptom of haemophilia at 6 years of age (bleeding after tooth extractions) Bleedings after external wounds and minor operations, and had received 10 blood transfusions. No haemarthrosis. He could attend school normally

V 4 (P.L.) Born 1946 died 1956 of haemophilia. He had always a bleeding tendency after wounds. No haemarthrosis.

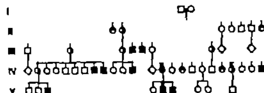
Family 72, Haemophilia A, severe form.

FAMILY 72

IV 10 (B.B.) Born 1940 First symptom of haemophilia at 9 months of age (haemarthrosis) Repeated haemarthroses in all the main joints, which have caused severe disability He cannot walk, is bound to a wheel-chair since 8 years of age. Hospitalized several times for haemarthrosis and renal haemorrhage and has received about 100 blood transfusions and the same number of AHF-preparations. He has private education at home four hours a week.

IV 11 (L.B.) Born 1948 First symptom of haemophilia at one year of age Repeated haemarthroses in all the main joints, which have caused moderate disability He is also bound to a wheel-chair Hospitalized several times for haemarthrosis, and has received about 40 blood transfusions and 30 AHF preparations. He has also private education at 4 hours a week.

Family 73, Haemophilia A, severe form.



III 4 (A.J.) Born 1891 died 1914 of pulmonary tuberculoses. He always bled easily

III 5 (L.J.) Born 1898, died 1912 of haemophilia. Repeated haemarthrosis, which caused severe disability

IV 7 Li ed i U.S.A. I said to have bled easily Died i early age after an accident.

IV 8 Lived in U.S.A. Bled easily died of postoperative haemorrhage

IV 11 Born 1918. Is said to be haemophiliac, lives in U.S.A.

IV 22 (A.J.) Born 1945. First symptom of haemoph lia at n year of age (haemarthrosis) Repeated haemarthroses, wh h have caused a stiff hip joi t and impaired func ti n f the knee, ankle and elbow joints. Hospitalized several times and has received about 20 blood transfusions. He could ttend or dinary school but was away long periods for haemarthrosis. He could not complet school courses.

V 3 Lives in U.S.A. Is said to be haemophiliac.

V 5 (J.O.) Born 1916, died 1959 of abdominal haemorrhage fter a wheel-chair accident. Repeated haemarthrosis, severely disabled. Hospitalized several times and recei ed about 70 blood transfusions.

V 6 (C.O.) Born 1948, died 1950 of excessive haemorrhage from a wound in the mouth.

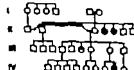
Family 74, Haemophilia A, severe form.



IV 8 (L.B.C.) Born 1931 F rst symptom of haemophilia at ne year of age (subcutaneous ha morrhages) Repeated haemarthroses all the main joints, which have caused moderate disability Hospitalized seve al times for haemarthrosis, gastrointestinal, renal and once intracranial haemorrhage, and has recei ed bout 50 blood transfusions and 10 AHF-preparations. H has private education at home.

IV 10 (L.C.) Born 19.., died 1956 of gastrointestinal haemorrhage. He bled easily after wounds and received 4 blood transfusions.

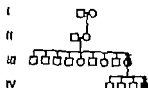
Family 75, Haemophilia A, severe form.



IV: 8 (L.T.) Born 1952. First symptom of haemophilia t 8 months of age (subcutaneous haemorrhage) A few times haemarthrosis in the ankle knee and lbow joints,

which has not impaired the joint function Hospitalized four times for haemathrosis and has received one blood transfusion. He can at tend school normally but is free from gymnastics.

Family 76, Haemophilia A, severe form.



IV 4 (L.S) Born 1942. First symptom of haemophilia at five years of age (haemarthrosis) Repeated haemarthroses in all the main joints which have caused impaired function of the left knee joint. Hospitalized several times for haemarthrosis, renal and once gastrointestinal haemorrhage, and has received about 30 blood transfusions. He could attend school fairly normally but one year he attended a special school for handicapped children. He is a factory worker

F mily 77 Haemophilia A, severe form.



IV 3 (T.S) Born 1949 First symptom of haemophilia at 4 months of age (haemorrhage after dentition) Repeated haemarthroses in the ankle knee and elbow joints, which have caused moderately impaired function of the right elbow joint. Hospitalized a few times for haemarthrosis and once for renal haemorrhage and has received about 75 blood transfusions. He can attend ordinary school but is free from gymnastics.

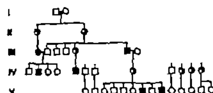
IV 4 (S.S.) Born 1955 First symptom of haemophilia at 8 months of age (subcutaneous haemorrhage) He has not yet had any haemarthrosis. Hospitalized once for intramuscular haemorrhage and has received 7 blood transfusions.

Family 78, Haemophilia A, severe form.



IV 6 (B.E.) Born 1955. First symptom of haemophilia at birth (umbilical haemorrhage) Repeated haemarthroses in the main joints, which have not yet impaired the joint function. He also has cerebral palsy Hospitalized several times for haemarthrosis, and has received about 20 blood transfusions.

Family 79, Haemophilia, clinically mild form.



III 6 (N.O.) Born 1881. Is said to have bled easily after wounds.

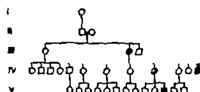
IV 2 (J.O.) Born 1910, died 1953 after suicidium. He is said to have bled easily after wounds and tooth extractions.

IV 5 (A.M.) Born 1922. Is said to have bled easily after wounds and tooth extractions.

V 7 (S.J.) Born 1933. First symptom of haemophilia at 13 years of age (excessive haemorrhage after tooth extraction). No haemarthrosis. Hospitalized two times for haemorrhage after tooth extraction and alienotomy (dentists drill) and has received about 20 blood transfusions. He could attend school normally, has been in military service and works as a lumberman.

V 8 (B.J.) Born 1938. First symptom of haemophilia at 15 years of age (excessive haemorrhage after tooth extraction). No haemarthrosis. Hospitalized once for haemorrhage after tooth extraction and has received one blood transfusion. He could attend school normally, has been in military service and works as a lumberman.

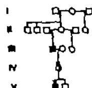
Family 80, Haemophilia A, severe form.



IV 12 (L.N.) Born 1936. First symptom of haemophilia at three years of age (bleeding from a wound in the mouth). Repeated haemarthroses, which has caused an impaired function of the left knee joint. Hospitalized several times for haemarthrosis and renal haemorrhage and has received about 100 blood transfusions. He could attend school normally and is free from military service. He is studying.

V 11 (B.P.) Born 1958. First symptom of haemophilia at one month of age (after calmette vaccination). He has had a few haemarthrosis in the hip and elbow joints and has received 3 blood transfusions.

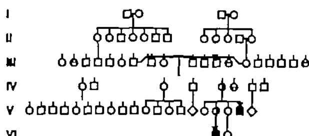
Family 81, Haemophilia A, moderate form.



III 1 (G.A.) Born 1901, died 1914 of intracranial haemorrhage. In youth he bled easily from wounds and in the joints after blows. He had impaired joint function.

V 1 (U.J.) Born 1951. First symptom of haemophilia at three years of age (haemarthrosis). Repeated haemarthroses in all the main joints, which have not yet caused any impaired function of the joint. Hospitalized a few times for haemarthrosis, and has received about 5 blood transfusions. He can attend school normally and is free from gymnastics.

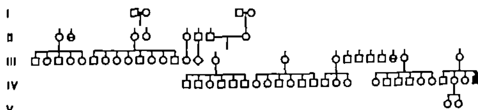
Family 82, Haemophilia A, severe form.



V 18 (E.W.) Born 1933. First symptom of haemophilia at three years of age (haematoma in the throat). Repeated haemarthroses, which have caused a stiff right knee and impaired function of the other knee and elbow joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhage, operated for appendicitis (excessive haemorrhage from the operation area) and has received about 20 blood transfusions. He could attend ordinary school but was away long periods due to haemorrhages. Is free from military service, has no occupation and lives on disability pension.

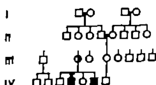
VI 1 (R.H.) Born 1950. First symptom of haemophilia at one year of age (excessive haemorrhage after a wound). Repeated haemarthroses, which have caused impaired function of the ankle joints. Hospitalized a few times for haemarthrosis and has received about 30 blood transfusions. He can attend ordinary school, but is away several days due to haemarthrosis.

Family 83, Haemophilia A, moderate form.



IV 25 (P.O.) Born 1916. First symptom of haemophilia at six months of age (haematoma in the head). Repeated haemarthroses in the ankle knee and elbow joints, which have not yet caused any impaired joint function. Treated in hospital several times for haemarthrosis and once for renal haemorrhage, and has received about 30 blood transfusions. He can attend ordinary school, but the last year he has been away most of the time.

Family 84, Haemophilia A, severe form.

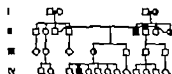


IV 4 (R.H.) Born 1938. First symptom of haemophilia at two years of age (haemorrhage after an accident). Repeated haemarthroses in the ankle knee elbow and wrist joints which have not yet caused any impaired function of the

joints. Hospitalized several times for haemarthrosis, once for renal and 1 tracranial haemorrhage, and has received about 80 blood transfusions. He could attend school fairly normally and is free from military service He works as a farmer

IV 6 (H.H.) Born 1940 died 1942 of haemophilia. He always bled easily after blows and wounds.

Family 85, Haemophilia A, severe form.



II 5 (K.F.) Born 1881 died 1887 probably of intracranial haemorrhage. He always bled easily

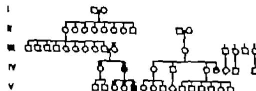
IV 4 (B.C.) Born 1918. First symptom of haemophilia t one year of age (haemarthrosis) Repeated haemarthroses in all the main joi ts, which have caused a stiff knee joint. Hospitalized several times for haemarthrosis, renal and gastroi testinal haemorrhage and has received about 60 blood transfusions and once AHF-preparation. H attends ordinary school, but is away most of the time.

Family 86, Haemophilia A, severe form.



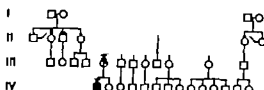
IV 9 (G.S.) Born 1915. First symptom of h emophilia t eight months of ge (haemarthrosis) Repeated haemarthroses n all the mai joints, whi h have caused a stiff right knee nd impaired function of the left knee Hospitalized se eral times for haemarthrosis and gastrointestinal haemorrhage, and has recei ed about 60 blood transfusions. He has private education at home

Family 87, Haemophilia A, moderate form.



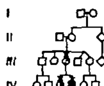
V 5 (R.F.) Born 1919. First symptom of haemoph li fter birth (subcutaneous haemorrhage) A few haemarthroses, the ankle knee and wrist joi ts, which have not yet caused any impaired joint function. Hospitalized a few times for haemarthrosis, renal haemorrhge and appendectomy which was performed under protection of totally 7 AHF-preparations. He has received about 6 blood transfusions. He can attend school normally but is free from gymnastics.

Family 88, Haemophilia A, severe form.



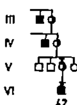
IV 1 (R.K.) Born 1945. First symptom of haemophilia at three months of age (haematoma). Repeated haemarthrosis in the ankle, knee elbow and wrist joints, which have caused moderately impaired function of the knee and elbow joints. Hospitalized several times for haemarthrosis, renal and gastrointestinal haemorrhages and has received about 150 blood transfusions. He can attend ordinary school, but is free from gymnastics, but is away from school fairly often.

Family 89. Haemophilia A, severe form.



IV 4 (K.A.) Born 1943. First symptom of haemophilia at 7 months of age (haematoma after an accident). Repeated haemarthrosis in the main joints, especially the ankle, knee, elbow and wrist joints, but without any impaired joint function. Hospitalized several times for haemarthrosis and renal haemorrhage, and has received about 50 blood transfusions. He could attend ordinary school, but is away long periods of time. He is now an office apprentice.

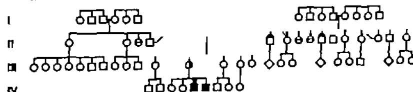
Family 90, Haemophilia A severe form.



The family is of Finnish origin and described by Ikkala (1960) under family A I 2. An extract of the original pedigree published by Ikkala is presented here.

IV 42 (Ikkala VI 42) (P.B.) Born 1956. First symptom of haemophilia at one year of age (haematoma in the head). Repeated haemarthrosis in all the main joints, which has not yet caused any impaired joint function. Hospitalized a few times for haemarthrosis and has received about 10 blood transfusions.

Family 91, Haemophilia A, severe form.

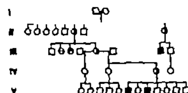


IV 5 (S.G.) Born 1952. First symptom of haemophilia at six months of age (haemorrhage after otitis media). Repeated haemarthroses in all the main joints, which have not yet caused any impaired joint

function. Hospitalized a few times for haemarthrosis and intracranial haemorrhage after which he recovered but has cramps now and then. He has received about 20 blood transfusions. He can attend ordinary school, but is free from gymnastics.

IV 6 (M.G.) Born 1960. First symptom of haemophilia after birth (umbilical haemorrhage). After bruises h bleeds easily.

Family 92, Haemophilia, clinically severe form.

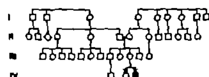


III 5 (X.X.) Died of excessive haemorrhage i youth.

V 6 (B.F.) Born 1912. First symptom of haemophilia at one year of age (bleeding in the mo th after n accident). Repeated haemarthroses in the ankle knee elbow and wrist joint, which have caused impaired function of the knee and elbow joints. Hospitalized a few times for haemarthrosis and bleedings from wounds in the mouth, and has received about 10 blood transfusions. He could attend ordinary school fairly normally d work as an assistant t photographer.

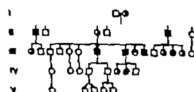
V 8 (B.L.F.) Born 1918 died 1946 of intracranial haemorrhage at six months of ge.

Family 93, Haemophilia A, mild form.



IV 3 (I.C.) Born 193.. First symptom of haemophilia t years of age (excess haemorrhage after a cut wound). A few times haemarthroses in the main joints, which have not impaired the joint function. Hospitalized few times for haemarthrosis and excessive haemorrhage after tooth extractions, and has received about 10 blood transfusions. He could attend school normally is free from military service and works as a farmer.

Family 94, Haemophilia A, mild form.



II 1 (A.A.) Born 1857 died 1932. He always bled easily.

II 5 (O.L.) Born 1870, died 19.5. He always bled easily repeated gastrointestinal haemorrhages.

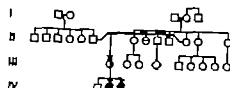
III 7 (A.K.) Born 1893. First symptom of haemophilia at 17 years of age (excessive haemorrhage after tooth xtraction). No haemarthrosis. Hospitalized a few times for xcess haemorrhage after tooth extractions, minor surgery and gastrointestinal haemorrh-

ages, he has received about 40 blood transfusions. He works as a clerk.

III 8 (G.K.) Born 1897 died 1947 of gastrointestinal *haemorrhage*. He always bled easily.

III 10 (K.K.) Born 1903. He has always bled easily after wounds and minor surgery. No haemarthrosis.

Family 95, Haemophilia A, severe form.



IV 2 (B.M.) Born 1946. First symptom of haemophilia at one year of age (subcutaneous haemorrhage). Repeated haemarthroses in all the *main joints, which have caused* impaired function of the ankle knee and elbow joints. Hospitalized several times for haemarthrosis and has received about 100 blood transfusions. He can attend ordinary school, is free from gymnastics, but is away from school long periods of time.

IV 3 (L.M.) Born 1954. First symptom of *haemophilia* at one year of age (hemorrhage at dentition). A few times haemarthrosis in the main joints, which has not yet caused any impaired joint function. Hos*pitalized a few times and has* received about 10 blood transfusions.

Family 96, Haemophilia A, mild form.



IV 5 (R.A.) Born 1943. First symptom of haemophilia at 5 years of age (haemorrhage after a wound in the mouth). A few times haemarthrosis in the ankle joints, but no impaired joint function. Hospitalized a few times for excessive haemorrhage after minor surgery and has received about 10 blood transfusions. He can attend ordinary secondary school.

Family 97, Haemophilia A, moderate form.

FAMILY 97



There has not been possible to *get any information* about the family because the patient is adopted and nothing is known about the parents.

II 1 (R.F.) Born 1920. First symptom of haemophilia at 7 years of age (haemorrhage after tooth extraction). Repeated haemarthroses in the ankle knee and elbow joints, which have moderately impaired the function of left knee joint. Hospitalized several times for haemarthrosis, renal and gastro*intestinal haemorrhage and has* received about 100 blood transfusions. He could attend school fairly normally and is free from military service. He works as a clerk.

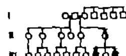
Family 98, Haemophilia A, mild form.



It has not been possible to obtain any informations about the family due t oligophrenia. As far it is known there is no bleeding tendency in the f mily

II 1 (R.J.) Born 1924 First symptom of haemophil a t 7 years of ge Repeated haemarthrosis in the ankle knee and elbow joints. 1934 both knees were operated after haemarthrosis, the left knee is stiff the right knee has moderately impaired function. Hospitalized a few times for haemarthrosis, renal and gastrointestinal haemorrhages, and has received about 10 blood transfusions. He could attend school normally is free from military service and ha no occupation lives on disabilit pension

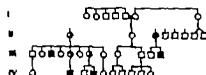
Family 99 Haemophilia B, severe form.



III 8 (R.C.) Born 1939 died 1960 f i terior ha morrhage. First symptom f haemophilia at two years f age (haematoma after an accident) Repeated haemarthrosis in ll the main joints, which did not cause ny impaired joint fun tion. Hospitalized several times for haemarthrosis, and has rece ed about 50 blood transfusi ns. He could attend school fairly normally and was free from military service. He worked as clerk in a store.

III 10 (G.G.) Born 1947 First symptom of haemophilia at one year of age (haematoma after an accident) Repeated haemarthroses n th nkle and knee joints, which have n t yet caused ny impaired joint function Hospitalized a few t mes for haemarthrosis and has received about 30 blood transfusions. He can attend school normally nd is free from gymnastics.

Family 100, Haemophilia B, severe form.



III 3 (B.W.) Born 1914 First symptom f haemophilia at two years of ge Repeated haem rthroses n all the main joints, which ha caused moderat ly impaired function of the nkle knee shoulder nd elbow joints. Hospitalized f w times for haemarthrosis nd renal haemorrhage he has received about 5 blood transfusions. He could attend school fai ly normally i free from military service nd works as a watchmaker

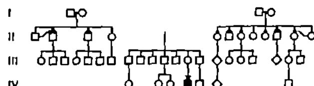
III 12 (R.N.) Born 1920 died of excessive haemorrhage after tooth ex tracti n in youth. He always bled easily after wounds.

IV 2 (S.B.W.) Born 1916 died 1918 of *haemophilia. H alway bled easily*

IV 4 (B.A.) *Born 1918. First* symptom f haemophilia at 7 months of age (subcutaneous haemorrhage)

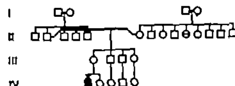
Repeated haemarthroses in all the main joints, which have caused moderately impaired function of the knee joints. Hospitalized once for haematoma in the bucca but has never received any blood transfusions He can only attend school sporadically

Family 101 Haemophilia B, moderate form.



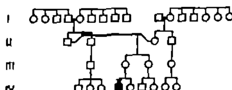
IV 4 (K.G.J) Born 1944 First symptom of haemophilia at 6 years of age (subcutaneous haemorrhage) Once haemarthrosis in the knee joint but no impaired function Hospitalized a few times for haemorrhages after wounds and intramuscular haematomata, and has received about 10 blood transfusions. He could attend school normally and works as a delivery man

Family 102, Haemophilia A, mild form.



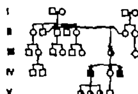
IV 1 (H.J H) Born 1940. First symptom of haemophilia at one year of age (subcutaneous haemorrhage) A few times haemarthrosis in the ankle knee, elbow and wrist joints, which have not caused any impaired joint function Hospitalized a few times for minor surgery but have not received any blood transfusions. He can attend school normally but is free from gymnastics.

Family 103, Haemophilia A, severe form.



IV 4 (L.G.J) Born 1947 First symptom of haemophilia at 8 months of age (haemarthrosis) Repeated haemarthroses in all the main joints, which have caused severe disability both in the arms and the legs. He is bound to a wheel chair Hospitalized several times for haemarthrosis, renal and gastrointestinal haemorrhages and has received about 180 blood transfusions. He attends a special school for handicapped children.

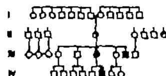
Family 104, Haemophilia A, mild form.



IV 3 (N N) Born 1926. First symptom of haemophilia at 8 years of age (tonsillectomy) No haema throsis. Hospitalized few times fter minor surgery and has received about 5 blood transfusions. He ould ttend school normally and is a drafted army officer He works as n ngineer

IV 5 (J V) Born 1937 First symptom of haemophilia at 10 years of age (renal haemorrh ge) N haemar throsis. Hospitalized a few times for intramuscular haematoma and renal haemorrhages, and has recei ed about 10 blood transfusions. He could ttend school nor mally and is free from military service. He works as an engineer

Family 105, Haemophilia A, severe form.



III 3 (A.N) Born 1934 First symptom f haemophilia t 6 years of ge (subcutaneous haemo rhage) No haemarthrosis. Bleed easily fter woun ls. Neve hospitalized. He could attend school normally and is free from military service. He works as a carpenter

IV 6 (U.B.) Born 1950 First symptom of haemophilia at 7 years of age (subcutaneous haemorrhages) A few times haemarthroses in the ankle and wrist joints, whi h have not impaired the joint function. Hospitalized few times for hae marthrosis and subcutaneous haemorrhages, and has received ne blood transfusion.

Family 106, Haemophilia A, severe form.



III (S.O.J) Born 1955. First symptom of haemophilia at two years of age (subcutaneous haemorrhage) He has had on haemarthrosis in the elbow joint, no impaired func tion. Hospitalized for haemorrhages from wounds a d has recei ed about 10 blood transfusions and three AHF-preparations.

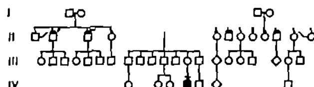
Family 107 Haemophilia A, mild form.



II 1 (S.O) Born 192 . First symptom of haemophilia t 20 years of age (excessive haemorrhage after tooth extraction) N haemar throsis. Hospitalized once for haemorrhages after tooth extraction, but received no blood transfusions. He has been in military servic and works s an excavator repairman.

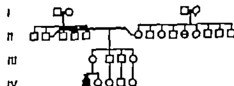
Repeated haemarthroses in all the main joints which have caused moderately impaired function of the knee joints. Hospitalized once for haematoma in the bucca, but has never received any blood transfusions He can only attend school sporadically

Family 101, Haemophilia B, moderate form.



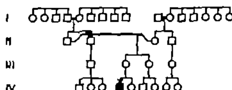
IV 4 (K.C.J.) Born 1944 First symptom of haemophilia at 6 years of age (subcutaneous haemorrhage) Once haemarthrosis in the knee joint but no impaired function. Hospitalized a few times for haemorrhages after wounds and intramuscular haematomata and has received about 10 blood transfusions. He could attend school normally and works as a delivery man.

Family 102, Haemophilia A, mild form.



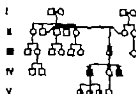
IV 1 (H.J.H.) Born 1940 First symptom of haemophilia at one year of age (subcutaneous haemorrhage) A few times haemarthrosis in the ankle knee, elbow and wrist joints, which have not caused any impaired joint function Hospitalized a few times for minor surgery but have not received any blood transfusions. He can attend school normally but is free from gymnastics.

Family 103, Haemophilia A, severe form.



IV 4 (L.G.J.) Born 1947 First symptom of haemophilia at 8 months of age (haemarthrosis) Repeated haemarthroses in all the main joints, which have caused severe disability both in the arms and the legs. He is bound to a wheelchair Hospitalized several times for haemarthrosis renal and gastrointestinal haemorrhages and has received about 180 blood transfusions. He attends a special school for handicapped children.

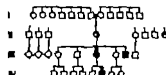
Family 104, Haemophilia A, mild form.



IV 3 (N.V.) Born 1926. First symptom of haemophilia at 8 years of age (tonsillectomy). No haemarthrosis. Hospitalized a few times after minor surgery and has received about 5 blood transfusions. He could attend school normally and is a drafted army officer. He works as an engineer.

IV 5 (J.N.) Born 1937. First symptom of haemophilia at 16 years of age (renal haemorrhage). No haemarthrosis. Hospitalized a few times for intramuscular haematoma and renal haemorrhages, and has received about 10 blood transfusions. He could attend school normally and is free from military service. He works as an engineer.

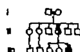
Family 105, Haemophilia A severe form.



III 5 (A.N.) Born 1934. First symptom of haemophilia at 6 years of age (subcutaneous haemorrhage). No haemarthrosis. Bleeds easily after wounds. Never hospitalized. He could attend school normally and is free from military service. He works as a carpenter.

IV 6 (U.B.) Born 1950. First symptom of haemophilia at 7 years of age (subcutaneous haemorrhages). A few times haemarthroses in the ankle and wrist joints, which have not impaired the joint function. Hospitalized a few times for haemarthrosis and subcutaneous haemorrhages, and has received one blood transfusion.

Family 106, Haemophilia A, severe form.



III 5 (S.O.J.) Born 1955. First symptom of haemophilia at two years of age (subcutaneous haemorrhage). He has had a haemarthrosis in the elbow joint, no impaired function. Hospitalized for haemorrhages from wounds and has received about 10 blood transfusions and three AHF-preparations.

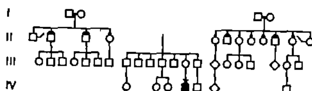
Family 107 Haemophilia A, mild form.



II 1 (S.O.) Born 1925. First symptom of haemophilia at 30 years of age (excessive haemorrhage after tooth extraction). No haemarthrosis. Hospitalized once for haemorrhages after tooth extraction, but received no blood transfusions. He has been in military service and works as an excavator repairman.

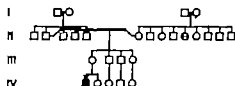
Repeated haemarthroses in all the main joints, which have caused moderately impaired function of the knee joints. Hospitalized once for haematoma in the bucca, but has never received any blood transfusions. He can only attend school sporadically

Family 101, Haemophilia B, moderate form.



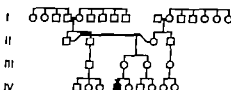
IV 4 (K.G.J.) Born 1944. First symptom of haemophilia at 6 years of age (subcutaneous haemorrhage). Once haemarthrosis in the knee joint but no impaired function. Hospitalized a few times for haemorrhages after wounds and intramuscular haematomata and has received about 10 blood transfusions. He could attend school normally and works as a delivery man.

Family 102, Haemophilia A, mild form.



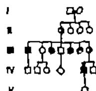
IV 1 (H.J.H.) Born 1940. First symptom of haemophilia at one year of age (subcutaneous haemorrhage). A few times haemarthrosis in the ankle, knee, elbow and wrist joints, which have not caused any impaired joint function. Hospitalized a few times for minor surgery but have not received any blood transfusions. He can attend school normally but is free from gymnastics.

Family 103, Haemophilia A, severe form.



IV 4 (L.G.J.) Born 1947. First symptom of haemophilia at 8 months of age (haemarthrosis). Repeated haemarthroses in all the main joints, which have caused severe disability both in the arms and the legs. He is bound to a wheelchair. Hospitalized several times for haemarthrosis, renal and gastrointestinal haemorrhages and has received about 180 blood transfusions. He attends a special school for handicapped children.

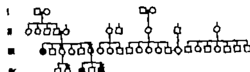
Family 111, Haemophilia A, moderate form.



III 1 (O.D.) Born 1883 died 1908 of excessive haemorrhage after appendectomy. H had repeated haemarthrosis and bled easily after tooth extractions. H worked as a sailor.

IV 1 (E.N.) Born 1929. First symptom of ha mophilia t 7 years of age (haemorrhage after tooth extraction). Repeated haemarthroses, which have caused impaired function of the left knee and right el bow joints. Hospitalized several times for haemorrthrosis and renal haemorrhages (30 times) and has recei ed about 50 blood transfusi ns and 6 AHF-preparations. H could attend school sporadically and is free from military service. He has disability pension and w rks as n editor f r small paper.

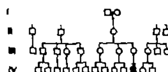
Family 112, Haemophilia B, severe form.



IV 3 (E.J.) Born 1931 died 1951 of haemophilia. Repeated haemar throsis and xcessive haemorrhage after tooth extractions and wound. H wa a factory worker.

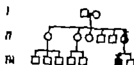
IV 5 (S.J.) Born 1943. First symptom f haemophili t six months of age (subcutaneous haemorrhages). Repeated haemarthroses in the ankle knee shoulder and elbow joints, which have caused impaired function of the left knee, shoulder and elbow joints. H is bound to wheel-chai Hospitalized several times for haemar throsis, renal and gastrointestinal haemorrhages, and has received about 100 blood transfusions. Could not ttend school but has had private education t home. H has no occupation and li es on disability pension.

Family 113, Haemophilia A, moderate form.



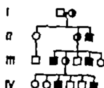
IV 11 (R.A.) Born 1939. First symptom of haemophilia t ne year of age (haematoma after an accid nt). He has had a few haemarthroses in the ankle knee nd hip joint which have not caused any impaired joint function. Hospitalized a few times for haemarthrosis and renal haemorrhages and has received 5 blood transfusions. He could attend ordinary school, is free from military service and works as a lerk.

Family 108, Haemophilia A, severe form.



III 6 (P.V.) Born 1945. First symptom of haemophilia at 8 months of age (haemorrhage from a wound in the lip). Repeated haemarthroses in all the main joints, which have caused impaired function of the knee joints. Hospitalized several times for haemarthrosis, and has received about 70 blood transfusions and 58 AHF preparations. He attends school normally but is free from gymnastics.

Family 109. Haemophilia, clinically mild form.



II 3 (A.F.) Born 1898, died of bleeding after appendectomy at an early age. He is said to have bled easily after wounds.

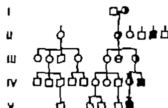
III 2 (U.P.) Born 1913. He has had excessive *haemorrhage after tooth* extractions. No haemarthrosis.

III 5 (S.P.) Born 1919. Repeated gastrointestinal haemorrhages. No haemarthrosis. *He always bleeds* easily after wounds.

IV 3 (C.A.) *Born 1943. First symptom of* haemophilia at 3 years of age (haematoma in the lip after an acci*dent). A few times haemarthroses* in the knee and wrist joints, which have not caused any impaired *joint function. Hospitalized a few* times for haemorrhages after cut wounds and tooth extractions, *and has received about 10 blood* transfusions. He can attend ordinary *school* but is free from gymnastics.

IV 6 (J.A.) Born 1953. He has always *bled easily after* wounds. No haemarthrosis.

Family 110, Haemophilia B, severe form.

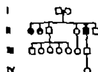


II 5 (O.A.) Born 1800 died *1867* of haemorrhage from the nose. He always bled easily.

IV 10 (H.F.) Born *1919* died *1930* of *intracranial haemorrhage. He al*ways bled easily after wounds and had often subcutaneous haemorrhages.

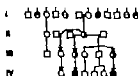
V 5 (J.H.) Born 1942. First symptom of haemophilia at one year of age (dentition). Repeated *haemar*throses in all the main joints which have caused impaired function of the knee *joints. Hospital*ized several times for haemarthrosis, gastrointenstinal and renal haemorrhages, and *has received* about 100 blood transfusions. He can attend ordinary school but is away *long periods of time* and is free from gymnastics.

Family 117 Haemophilia B, mild form.



II 5 (N.L.) Born 1893, died 1960 of intracranial haemorrhage. First symptom of haemophilia at 35 years of age (excessive haemorrhage after abdominal operation) No haemarthrosis. Hospitalized twice for renal haemorrhage, but had not recieved any blood transfusions. He worked as a painter

Family 118, Haemophilia B, severe form.



IV 2 (C.P.) Born 1955. First symptom of haemophilia at 9 months of age (subcutaneous haemorrhages) Haemarthrosis once or twice in all the main joints, which have not yet caused any impaired joint function. Hospitalized a few times for haemarthrosis and haemorrhage from a wound in the tongue and has received about 5 blood transfusions.

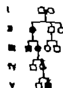
IV 6 (R.P.) Born 1958, died 1959 of intracranial haemorrhage First symptom of haemophilia at 5 months of age (subcutaneous haemorrhages) No haemarthrosis.

Family 119, Haemophilia A, severe form.

It is not possibl to get any information about his family because th patient is on home for parentless children. The patient mother is adopted, and does not know her real parents.

(J.M.) Born 1956. First symptom f haemophilia t 7 months of ge (haem rrhage after cut in the heel for a blood sample disputed paternity case) Repeated haemorrhages from the nose and has narthroses i all the main joi ts, which have not yet caused any impaired function of the joints. Hospitalized several times for haemorrhages from the nose and after wounds, and has received about 10 blood transfusions.

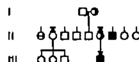
Family 120, Haemophilia A, severe form.



III 1 (A.S.) Born 1885, died 1918 of haemophilia. Repeated haemarthrosis, which caused severe disability He always bled easily from wounds.

V 2 (L.E.A.) Born 1957 First symptom of haemophilia at 10 months f age (subcutaneous haemorrhages) Excessive haemorrhage after a bit in the tongue. No haemarthrosis yet. Hospitalized once for haemorrhage after wound and has received on blood transfusion.

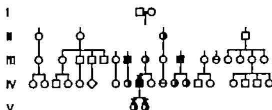
Family 114 Haemophilia A, severe form.



II 7 (T.J.) Born 1937 First symptom of haemophilia at one year of age (haematoma after trauma) Repeated haemarthroses in all the main joints, which have caused impaired function of the ankle knee and elbow joints He has difficulties in walking. Hospitalized several times for haemarthrosis and has received about 30 blood transfusions. He could attend school sporadically but was away half the time and is free from military service. He has no occupation and lives on disability pension

III 4 (B.J.) Born 1947 First symptom of haemophilia at 5 months of age (haematoma) Repeated nose bleedings after traumata no haemarthrosis. Hospitalized a few times for excessive haemorrhages after wounds, and has received about 5 blood transfusions.

Family 115, Haemophilia A, mild form

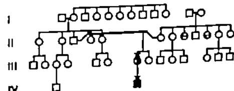


III 7 (S.P.) Born 1898, died 1950 of t.b.c. pulm. He bled easily after wounds and tooth extractions. No haemarthrosis. He was a factory worker and had silicosis pulm III

III 10 (B.P.) Born 1904 First symptom of haemophilia at 1? years of age (tooth extraction) He has always bled excessively after tooth extractions, once haemarthrosis in the right knee and a few times haematoma after injuries. Hospitalized a few times and has received about 50 blood transfusions. He could attend school normally and is free from military service. He is a craftsman

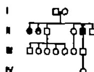
IV 8 (A.P.) Born 1922. First symptom of haemophilia at 33 years of age (excessive haemorrhage after tooth extraction) No haemarthrosis. A few times haematoma after injuries. Hospitalized two times after tooth extractions, and has received about 5 blood transfusions. He could attend school regulary has been in military service and works as a manager in a grocery store

Family 116, Haemophilia B, severe form.



IV 2 (U.O.) Born 1956. First symptom of haemophilia at 9 months of age (subcutaneous haemorrhage) Haemarthrosis two times in the ankle joint, which have not yet caused impaired of function of the joint. Hospitalized once and has received 2 blood transfusions.

Family 117 Haemophilia B, mild form.



II 5 (N.L.) Born 1892, died 1960 of intracranial haemorrhage. First symptom of haemophilia at 36 years of age (excessive haemorr hage after abdominal operation) No haemarthrosis. Hospitalized twice for renal haemorrhage, but had not receleved any blood transfusions. H worked as a painter

Family 118, Haemophilia B, severe form.



IV 2 (C.P) Born 1955. First symptom of haemophilia t 9 m nths of ge (subcutaneous haemorrhages) Haemarthrosis o ce or twice in all the main joints, which have not yet caused any impaired joint function. Hospitalized a few times for haemarthrosis and haemorr hage from a wound in the to gue nd has received about 5 blood transfusions.

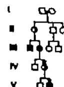
IV 6 (R.P) Born 1958, died 1959 of intracranial haemorrhage. First symptom of haemophilia at 0 mo ths of age (subcutaneous haemorrhages) No haemarthrosis.

Family 119 Haemophilia A, severe form.

It is n t possible t get any information about his family because the patient is on a home for parentless children. The patient mother is dopted, nd does not know her real parents.

(J.M.) Born 1956. First symptom of haemohilia at 7 m ths of age (haemorrhage after cut in the heel for a blood sample, disputed paternity case) Repeated haemorrhages from the nose and haemarthroses i all the main joints, which have not yet caused any impaired function of th joints. Hospitalized several times for ha morrhages from the nose and after wounds, and has received about 10 blood transfusions.

Family 120, Haemophilia A, severe form.



III 1 (A.S.) Born 1885, died 1918 of haemophilia. Repeated haemar throsis, which caused severe disability He alw ys bled easily from wounds.

V 2 (L.E.A.) Born 1957 First symptom of haemophilia at 10 months of age (subcutaneous haemorr hages) Excessive haemorrhage fter a bit in the tongue. No haemarthrosis yet. Hospitalized once for haemorrhage afte a wound and has received one blood transfusion.

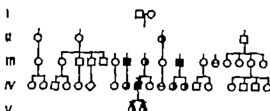
Family 114 Haemophilia A, severe form.



II 7 (T.J.) Born 1937 First symptom of haemophilia at one year of age (haematoma after trauma) Repeated haemarthroses in all the main joints, which have caused impaired function of the ankle knee and elbow joints. He has difficulties in walking. Hospitalized several times for haemarthrosis and has received about 30 blood transfusions. He could attend school sporadically but was away half the time, and is free from military service He has no occupation and lives on disability pension.

III 4 (B.J.) Born 1947 First symptom of haemophilia at 5 months of age (haematoma) Repeated nose bleedings after traumata no haemarthrosis. Hospitalized a few times for excessive haemorrhages after wounds, and has received about 5 blood transfusions.

Family 115, Haemophilia A, mild form

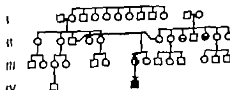


III 7 (S.P.) Born 1898 died 1950 of t.b.c. pulm He bled easily after wounds and tooth extractions. No haemarthrosis. He was a factory worker and had silicosis pulm III

III 10 (B.P.) Born 1904 First symptom of haemophilia at 12 years of age (tooth extraction) He has always bled excessively after tooth extractions, once haemarthrosis in the right knee and a few times haematoma after injuries. Hospitalized a few times and has received about 50 blood transfusions. He could attend school normally and is free from military service. He is a craftsman

IV 8 (A.P.) Born 192_ First symptom of haemophilia at 33 years of age (excessive haemorrhage after tooth extraction) No haemarthrosis. A few times haematoma after injuries. Hospitalized two times after tooth extractions, and has received about 5 blood transfusions. He could attend school regularly has been in military service and works as a manager in a grocery store

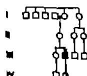
Family 116, Haemophilia B, severe form.



IV 2 (U.O.) Born 1956. First symptom of haemophilia at 9 months of age (subcutaneous haemorrhage) Haemarthrosis two times in the ankle joint, which have not yet caused impaired of function of the joint. Hospitalized once and has received 2 blood transfusions.

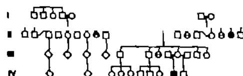
wound in the mouth) H has always bled xcessively from wounds and after tooth extractions. Hospitalized a few times for wound haemorrhages and haemarthrosi in the ankle knee and elbow joints, which have n t yet caused any impaired joint function. He has received about 10 blood transfusions and 4 AHF preparations. He can attend ordinary school.

Family 124, Haemophilia B, moderate form.



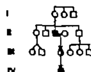
III 2 (A.H.) Born 1930 First symptom of haemophilia t 3 years of age (tooth extraction) Repeated haemarthroses in th knee hip d elbow joints, which have caused impaired function of the knee and right lbow joint. Hospitalized several times for haemarthrosis, renal a d gastrointestinal haemorrhages, and has received about 40 blood transfusions. H could ttend school normally is free from military service and worked s a bus driver but has now disability pension. He can only work sporadically

Family 125, Haemophilia, clinically mild form.



IV 7 (S.A.) Born 1939 First symptom of ha mophilia t 7 years of ge (haemorrhage aft r abrasion) A few times haemarthroses in the knee joi ts, whi h have not caused ny impaired joint fun tion. Hospitalized f excessive haemorrhages fter tooth extraction, and has recei ed about 5 blood transfusions. He could attend school normally and is free from mili tary service H works as a farme

Family 126, Haemophilia A, mild form.



II 3 (S.H.) Born 1895 died 1943 of gastrointestinal haemorrhage H lway bled easily after wound nd tooth extractions.

IV 1 (L.J.) Born 1957 First symptom of haemophilia at one year of age (subcutaneous haemorrhage) N haemarthrosis. He has not yet been hospitalized and has not re cei ed any blood transfusi s.

Family 121, Haemophilia A, severe form.

FAMILY 121

I
II
III
IV
V

III 4 (K.L.) Born 1908 died 1920 of haemophilia. *He always bled* easily and had repeated haemar throsis.

IV 4 (S.H.) Born 1926 died 1928 of haemophilia. He always bled easily

IV 10 (J H) Born 1935 First symptom of haemophilia at 2 yeras of *age (subcutaneous haemorhages)* Repeated haemarthrosis in the ankle knee and elbow joints, which have caused impaired function of the right elbow joint. *Hospitalized several times for* haemarthrosis, and haemorrhages after accidents. *He* has been appendectomized and has received totally about 25 blood transfusions. *He could attend school nor* mally is free from military ser vice and works as an auto electrician

Family 122, Haemophilia A, mild form.

FAMILY 122

I
II
III
IV
V

IV 6 (K.B.) Born 1918 First symptom of haemophilia at 7 years of age (tooth extraction) Repeated hae*marthroses in all the main joints*, which have not impaired the joint function Hospitalized several times for gastrointestinal and renal haemorrhages and has received *about 100 blood transfusions*. He could attend school normally is free from military service and works as a whole-sale dealer

IV 4 (R.B) Born 1915. First symptom of haemophilia at 15 years of age (tooth extraction) No haemar *throsis. Hospitalized once for ex* cessive haemorrhage after a hernia operation and received 3 blood transfusions. He has been in military service and works as hospital *superintendent*.

IV 7 (E.B) Born 1920 First symptom of haemophilia at *18* years of age (renal haemorrhage) No haemar throsis. Excessive haemorrhages after tooth extractions. Never hospitalized and has received no blood transfusions. *He has been* in military service and works as telegraph line repairman.

Family 123, Haemophilia A, mild form.

FAMILY 123

I
II
III
IV

II 5 (P.A.) Born 1883. He has always bled easily after wounds and tooth extractions.

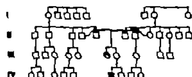
IV 4 (K.A) Born 1949 First symptom of haemophilia at one year of age (excessive haemorrhage from

Family 130, Haemophilia A, mild form.

It has not been possible to get a y information about this family

(K.E.P.) Born 1930. First symptom of haemophilia at 21 years of age (excessi e haemorrhage after appendectomy and he received about 30 blood tra sfusions) No haemarthrosis or other manifestations of bleeding ten dency H could attend school normally has been in military service and works as a w lder

Family 131, Haemophilia A, moderate form.

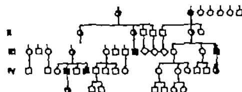


IV 6 (H.S.) Born 1955 First symptom of haemophilia at 10 months of age (haemorrhage from a wound in the mouth) Repeated haemar throses in the ankle knee shoulder elbow and wrist joints, which have not yet caused any impaired joint function. Hospitalized a few times for haemarthrosi nd has received about 5 blood transfusions.

Family 132, Haemophilia A, moderate form.

It has not been possibl to get any real information about the family because it comes from Esthonia. The patient can remember that he has had relati es on his mothers sid with bleed ng tendency
(A.M.) Born 1915. First symptom f haemophilia at 18 years of ge (xcessi e haemorrhage aft wound) No haemarthrosis, but intramuscular haemorrhages after blows. Hospitalized several times for renal haemorrhages, excessive haemorrhages fter appendect m and extirp tion f a colon polyp and has recei ed about 75 blood and plasma transfusions. He could attend school normally has been i military service and works as an engi eer

Family 133, Haemophilia A, mild form.

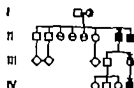


III 8 (N S) Born 1903, d ed 1930 f in terior haemorrhage. H always bled easily fter wounds and tooth extractions.

III 11 (S.B.) Born 1915. First symptom of haemophilia at 3 years of age (excessive haemorrhage after wound in the head) No haemarthrosis. Hospitalized several times for gastrointestinal haemorrhages nd has received about 120 blood transfusions. He could attend school normally and has been in military service He was formerly carpenter but works now as a radio and TV-dealer

IV 4 (S.G.K.) Born 1928. First symptom of haemophili at 2 years of age (excessive haemorrhage from a wound in the mouth) He has had a few haemarthroses in the knee joints without causing impaired function. Hospitalized a few times for haemorrhages after wounds nd tooth extractions

Family 127 Haemophilia A, mild form.

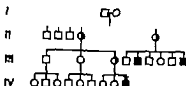


II 7 (A.P.) Born 1888. First symptom of haemophilia at 15 years of age (excessive haemorrhage after an accident) No haemarthrosis. Excessive haemorrhage after tooth extraction and surgery Hospitalized a few times for haemorrhages after surgery and has received about 50 blood transfusions and 7 AHF preparations. Worked as a fish dealer but is now retired.

II 8 (H.P.) Born 1893 died 1952 in mental hospital. He always bled easily from the nose and after tooth extraction.

IV 4 (C.F.) Born 1945 First symptom of haemophilia at one year of age (haemorrhage from a wound in the forehead) He has had two haemarthrosis in the hip joint. Hospitalized a few times for haemarthrosis and has received 6 blood transfusions. He can attend school normally

Family 128, Haemophilia A, moderate form.

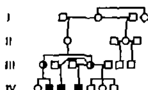


III 5 (T.J.) Born 1919 died 1927 of interior haemorrhage He had repeated haemarthrosis.

III 9 (I.J.) Born 1924 died 1935 of interior haemorrhage. He had repeated haemarthrosis and bled easily after wounds.

IV 9 (L.E.L.) Born 1942, died 1959 of gastrointestinal haemorrhage First symptom of haemophilia at one year of age (excessive haemorrhage after a wound) Repeated haemarthrosis in all the main joints, which caused impaired function of the ankle knee and wrist joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages and had received about 40 blood transfusions. He could attend school normally

Family 129, Haemophilia A, severe form.

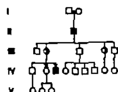


IV 2 (R.J.) Born 1949 First symptom of haemophilia at 6 years of age (subcutaneous haemorrhage) Repeated haemarthroses in the ankle, knee, elbow and wrist joints, which have not yet caused impaired joint function Hospitalized several times for haemarthrosis, and has received about 30 blood transfusions. He can attend ordinary school, but is free from gymnastics.

IV 3 (B.V.) Born 1913 died 1947 of intracranial haemorrhage He had haemarthrosis and bled easily after wounds.

IV 4 (H.S.) Born 1918, died 1950 of postoperative haemorrhage after brain surgery He had always bled easily after wounds.

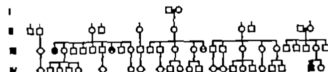
Family 137, Haemophilia A, mild form.



II 1 (C.A.) Born 1863 died 1935. He always bled easily after tooth extractions and wounds.

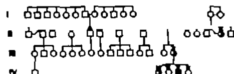
IV 3 (A.F.) Born 1935 First symptom of haemophilia at two years of age (excessi e ha morrhage after minor surgery) He has had a few haemarthrosis in the knee joints without causing impaired joint function. Hospitalized several times for haemorrhages after tooth extracti ns and has received about 10 blood transfusions. He could attend school normally and is free from military service. He works as a p inter

Family 138, Haemophilia A, moderate form.



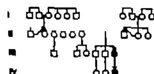
IV 16 (T.H.) Born 1942. First symptom of haemophilia at 2½ years of age (subcutaneous haemorrhages) Repeated haemarthroses in the ankle knee, hip, elbow and wrist joints, which have caused impair ed function of the right knee joint. Hospit l zed several times for haemarthrosis d haemorrhages after minor surgery and has recei ed about 20 blood transfusions. H could ttend six classes in the ordinary school, is free from military service a d has n occupation, lives on disablement pension

Family 139, Haemophilia B, mild form.



IV 4 (T.E.) Born 1917 First symptom of haemophilia t 3 years of ge (excessive haemorrhage after extraction of nail) He has had one haemarthrosis in the nkle and knee j i t without causing impaired joint function. Hospitalized few times for haemarthrosis and haemorrhages after mi or surgery and has received about 5 blood transfusions. He can attend ordinary school.

Family 140, Haemophilia A, severe form.



IV 3 (T.R.) Born 1936. First symptom of haemophilia t one year of age (subcuta eous haemorrhages) R peated haemarthroses i the nkle knee shoulder elbow and wrist joints, which have not yet caused

and has received about 5 blood transfusions. He could attend school normally *is free from military service* and works as a bank accountant.

IV 6 (A.K.) Born 1934 First symptom *of haemophilia* at 3 years of age (excessive haemorrhage from a wound in the lip) Repeated haemarthroses in the ankle and elbow which have not caused impaired function Hospitalized a few times for haemorrhages after tooth extractions. He could attend school normally Is free from military service and works as a driver instructor

Family 134, Haemophilia B, moderate form.



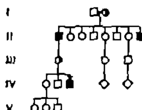
It has not been possible to get any further information about this family

I 2 Died of haemophilia in youth. He always bled easily after wounds.

II 1 Is said to have a tendency to bleed No other information available

II 3 *(S.N.) Born 1919 First* symptom of haemophilia at 5 years of age (excessive haemorrhage after tooth extraction) Repeated haemarthroses, which *have not* caused any impaired joint function. Hospitalized a few times for renal and gastrointestinal *haemorrhages* and has received about 50 blood transfusions. He could attend school normally and works as a manager at a service station

Family 135, Haemophilia A, mild form.



II 1 (J.N.) Born 1880 died 1902 of gastrointestinal haemorrhage He always bled easily after wounds and tooth *extractions.*

II 8 (N.N.) Born 1892, died 1905 of haemophilia *He always bled* easily

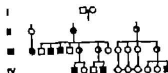
IV 3 (L.T.) Born 1937 First symptom of haemophilia at 3 years of age *(haemarthroses) Repeated haem*arthrosis in the ankle knee hip elbow and wrist joints, which has caused moderately impaired function of the right ankle joint. *Hospitalized several times for hae*marthrosis, renal and gastrointestinal haemorrhages and has received about 25 blood transfusions. He could attend ordinary school, but was away 2 years due to haemorrhages. He is free from *military service* and works as a clerk.

Family 136, Haemophilia A, moderate form.

Very little is known about the family It is not known if any other person in the family has a *bleeding tendency*

(G.P.) Born 1908. First symptom of haemophilia at 10 years of age (excessive haemorrhage after tonsillectomy) No haemarthrosis. *Hospitalized several* times for gastrointestinal haemorrhage and *haemorrhage after tooth* extractions, surgery for nephrolithiasis, and has received about 10 blood transfusions and 6 AHF preparations. He could attend school normally *is free from military* service and works as a storekeeper's assistant.

Family 144, Haemophilia A, moderat form.



III 3 (P.G.B.) Born 1890 died 1912 of excessi haemorrhage from an in cision in a haemat ma in the lip. He had repeated haemarthrosis and renal haemorrhages, received about 15 blood transfusions. He worked as a bicycle repairman.

III 4 (H.G.) Born 1902, died 1906. H always bled easily after wounds and in the j ints.

IV 1 (J.E.K.) Born 1941 First symptom of haemophilia at 2 years of age (haemarthrosis) Repeated haemarthroses in the nkle knee, hip elbow and wrist j ints, which have caused impaired function of the knee joints. Hospitalized several times for haemarthrosis and has received about 20 blood transfusions. H could attend ordinary school, b t was away one year for orthopaedic treatment of th legs. He is at a vocational training school to learn photography He has disablement pension.

IV 4 (B.N.) Born 1939 First symptom of haemophilia at two years of age (haemarthrosis) Repeated haemarthroses in ll th main j ints, which have caused impair ed function of the knee joints. Hospitalized several times for haemarthrosis and renal haemorr hages and has received about 30 blood transfusions. He could at tend school normally but was aw y lo g periods of time, nd is free from military service. He first tried t be a photographer b t could not finish the vocational training, is now a TV-repairman.

IV 7 (O.L.) Born 1946. First symptom of haemophilia at one year of age (excessive haemorrhage from wou d in the mouth) Repeated haemarthrosis in all the main j ints, which have caused impair ed f nction of the knee joints. Hospitalized a few times for ha marthrosis and has received about 15 blood transfusions. He can t tend school normally

Family 145, Haemophilia A, mild form.



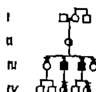
II 2 (A.H.) Born 1927 First symptom of haemophilia t 18 years of ge (excess haemorrhage after tooth extra tio) He has had no other bleedi g manifestation but excessi haemorrhage fter tooth tractions. He could attend school normally has been in military service and workes as a tractor driver

II 6 (H.H.) Born 1912. First symptom of haemophilia t 11 years of age (excessive haemorrhage after tooth extraction) \ haemarthrosis. He has bled excessively after tooth extractions and minor surgery but has n t received y blood transfusions. He could t tend school normally a d work as an aut repairman.

III 4 (K.H.) Born 1953. First symptom of haemophilia t 3 years of age (excessive wound haemorrhage) No haemarthrosis. Hospitalized once for haemorrhage after wounds but has not recei ed any blood transfusions.

any impaired joint function Hospitalized a few times for haemarthrosis and haemorrhages after an auto accident. He has received about 25 blood transfusions and 6 AHF preparations.

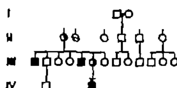
Family 141, Haemophilia A, mild form.



III 2 (O F) Born 1918. First symptom of haemophilia at 3 years of age (repeated nose bleedings) No haemarthrosis. Hospitalized a few times for haemorrhages after tooth extractions, laparatomy tonsillectomy and gastrointestinal haemorrhage and has received about 50 blood transfusions. He could attend school normally and has been in military service He works as an engineer

III 3 (L.F) Born 19?6 First symptom of haemophilia at 12 years of age (excessive haemorrhage after tooth extraction) No haemarthrosis. Hospitalized a few times for haemorrhages after tooth extractions, but has not received any blood transfusions. He attended school normally has been in military service and works as a precision building mechanic.

Family 142, Haemophilia A, severe form.

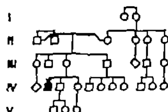


III 1 (S.F) Born 1909 died 1913 of interior haemorrhage after pertussis. He always bled easily after wounds and had haemarthroses.

III 5 (T.F) Born 1910 died 1930 of diphtheria Had haemarthroses and always bled easily after wounds.

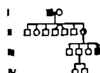
IV 2 (H.L.K.) Born 1946 First symptom of haemophilia at 3 months of age (excessive haemorrhages after operation of a hernia) Repeated haemarthrosis in all the main joints, which has caused impaired function of the knee joints. Hospitalized several times for haemarthrosis and haematoma in the muscles, and has received about ?0 blood transfusions. He can attend ordinary school, but has also received private education in the home when he has long periods of bleedings.

Family 143, Haemophilia A, severe form.



IV 1 (K.J) Born 19?3. First symptom of haemophilia at birth (haemorrhages from the anus) Repeated haemarthroses in the ankle knee hip elbow and wrist joints, which have caused *slightly impaired* function of the ankle knee and elbow joints. Hospitalized several times for haemarthrosis and renal haemorrhages and *has received* about 40 blood transfusions. He could attend school fairly normally is free from military service *and works as a repairman.*

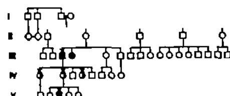
Family 149, Haemophilia A, mild form.



I 1 (A.L.) Born 1864 died 1934 H always bled easily after wounds. No haemarthrosis.

III 4 (A.S.) Born 1930 First symptom of haemophilia at 29 years of age (haemorrhage after incision of haematoma in the back) No haemarthrosis. Once a renal haemorrhage. He has not bled excessively fter tooth extractions. Hospitalized o ce for excessive haemorrhage after an operati n, and has received 3 blood transfusions. H could ttend school normally works as a petty officer in the Swedish navy

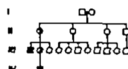
Family 150, Haemophilia B, moderate form.



III 3 (K.R.C.) Born 1907 First symptom of haemophilia at 7 years of ge (excessi haemorrhage after wound) N haemarthrosis. In youth he had frequent one-bleedings. Hospitalized a few times for haemorrhages after wound nd tooth extractions and has received 2 blood transfusi ns. H could ttend school normally has been in military service and works as a painter

V 3 (R.E.) Born 1955. First symptom of haemophilia t 11 months of ge (*subcutaneous haemorrhages*) Once haemarthrosis in the left knee joi t. Hospitalized a few times for haemorrhages after wounds, but has not received any blood transfusions.

Family 151, Haemophilia, clinically severe form.



III 1 (I.E.) Born 1933, died 1934 of haemophilia. First symptom f haemophilia t one yea of age (subcutaneous haemorrhages) He always bled easily after wounds. Hospitalized a few times for renal haemorrhage and haematoma.

IV 1 (T.K.) Born 1959 First sympt m of haemophilia t six months f ge (subcutaneous haemorrhages) N haemarthrosis yet. Bleed excessively after wounds. Hospitalized once for excessive haemorrhage from a wound in the upper lip and received 2 blood transfusions.

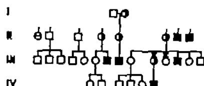
III 5 (K.H.) Born 1956 First symptom of haemophilia at 2 years of age (excessively wound haemorrhage) No haemarthosis. He has bled excessivly after tooth extractions and wounds.

Family 146, Haemophilia A, mild form.



III 2 (S.P.) Born 1928. First symptom of haemophilia at 17 years of age (excessive haemorrhage after tooth extractions) Hospitalized a few times for haemorrhages after tooth extractions and has received about 20 blood transfusions. He could attend school normally is free from military service and works as a moulder in a foundry

Family 147 Haemophilia A, severe form.



II 7 (S.K.) Born 1909 died 1947 of gastrointestinal haemorrhage. Repeated haemarthrosis which caused impaired function of the right knee and elbow joints. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages, and received about 10 blood transfusions. He worked as a farmer

II 8 (N.K.) Born 1912, died 1930 of haemorrhage after appendectomy He had repeated haemarthrosis.

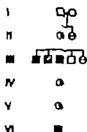
III 7 Born 1931 Lives in U.S.A. He has had repeated haemarthrosis and bleeds easily after wounds.

III 8 Lives in U.S.A. He has haemarthrosis and bleeds easily after wounds.

III 1. (N.E.P.) Born 1938 died 1941 of excessive haemorrhage from a wound in the lip He bled easily after wounds and had haematoma after light blows.

IV 6 (K.S.) Born 1961 First symptom of haemophilia at one month of age (subcutaneous haemorrhages)

Family 148, Haemophilia A, moderate form.



III 1 (H.A.) Born 1875, died 1889 of excessive haemorrhage after a tooth extraction He had haemarthrosis, and bled easily after wounds.

III 3 (I.A.) Born 1878, died 1909 of a haematoma in the throat. He always bled easily after wounds, had repeated haemarthrosis, which impaired the function of the knee joints. He worked as an artist.

VI 1 (P.W.) Born 1950 First symptom of haemophilia at 10 months of age (haemorrhage from a ruptured frenulum linguae) He easily has subcutaneous haemorrhages after light blows and has had a few haemarthroses. Hospitalized a few times for excessive haemorrhage from wounds and for haemarthrosis, and has received about 5 blood transfusions.

Family 155, Haemophilia A, moderate form.

FAMILY 155

I
II
III
IV

IV 5 (A.F.) Born 1954 First symptom of haemophilia at 6 months of ge (subcutaneous haem rrh ges) Repeated haemarthrosi in the ankl knee and elbow joints, which has n t caused any impair ed joi t function. Hospitalized se eral times for haemarthrosis and gastrointestinal haemorrhages and has received 3 blood transfusions.

Family 156, Haemophilia A, mild form.

FAMILY 156

I
II
III
IV
V

IV 5 (K.F.) Born 1953 First symptom f haemophilia t one year f age (subcutaneous haemorrhages) Repeated haemarthroses in the ankle, knee lbow nd wrist joints, which ha e not caused any impaired joint function. Hospitalized few times for haemarthrosis and gastrointestinal haemorrhages and has received 2 blood transfusions.

Family 157 Haemophilia A, mild form.

AMIL 157

III
IV

III 3 (O.B.) Born 1899 Excessive haemorrhages after tooth extractions and wounds. No haemarthrosis.

III 6 (A.M.) Born 1897 died 1920 of excessi e haemorrhage after a tooth extraction.

III 7 (E.J.) Born 1909. Excessi haemorrhages fter tooth extractions and wounds.

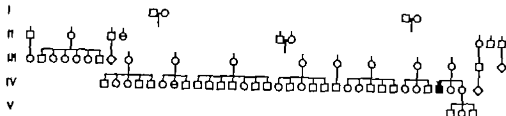
IV 3 (T.T.) Born 1919 Excessive haemorrhages fter tooth extractions and wounds. No haemarthrosis.

IV 12 (S.E.A.) Born 1949 H easily gets haematomas a d subcuta eous haemorrhages fter light blows.

V 2 (K.R.) Born 1918. H has bled easily after tooth extractio tonsillectomy and wounds. Hospitalized for ha morrhage after tonsillectomy and has received 5 blood transfusions. He can attend school normally

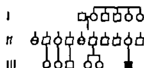
V 4 (J.E.R.) Born 1937 First symptom of haemophilia at 7 months of ge (excessive haemorrhage after rupture of frenulum linguae) No haemarthrosis. Hospitalized twice for haemorrhages after traumata and has received 2 blood transfusions.

Family 152, Haemophilia A, moderate form.



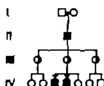
IV 30 (S.B.) Born 1918. First symptom of haemophilia at 3 years of age (nose-bleeding) No haemarthrosis. Hospitalized several times for nose-bleedings and haemorrhages after tooth extractions, and has received about 10 blood transfusions. He always bleeds easily after wounds. He could attend school normally is free from military service and is a textile worker

Family 153, Haemophilia B, moderate form.



III 6 (B.E.) Born 1945 First symptom of haemophilia at 2 years of age (subcutaneous haemorrhage) Repeated haemarthroses in the ankle, knee shoulder and elbow joints, which have caused slight impaired function of the knee joints. Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 10 blood transfusions. He can attend ordinary school, but is free from gymnastics.

Family 154, Haemophilia A, mild form.

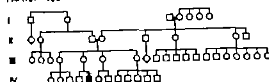


II 1 (E.J.) Born 1895 died 1935 of septicaemia He always bled easily after wounds and tooth extractions but had no haemarthrosis.

IV 3 (B.E.) Born 1947 First symptom of haemophilia at 5 years of age (excessive haemorrhage after abrasio) He easily has haematoma after blows. Hospitalized a few times, once for a haemarthrosis in the knee twice for haemorrhages after minor surgery and has received about 5 blood transfusions. He can attend school normally

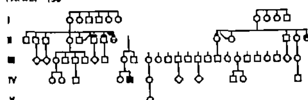
IV 4 (R.E.) Born 1950 First symptom of haemophilia at 5 months of age (excessive haemorrhage after a wound) He easily bleeds subcutaneously No haemarthrosis. Hospitalized once for haemorrhage from a wound but received no blood transfusions. He can attend school normally

Family 155, Haemophilia A, moderate form.



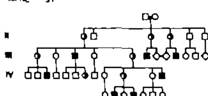
IV 5 (A.F.) Born 1954. First symptom f haemophilia at 6 months of ge (subcutaneous haemorrhages) Repeated haemarthrosis in the ankle knee nd elbow j ints, which has not caused any impair ed joint function. Hospitalized several times for haemarthrosis and gastrointestinal haemorrhages and has received 3 blood transfusions.

Family 156, Haemophilia A, mild form.



IV 5 (K.F.) Born 1953. First symptom f haemophilia at e year of age (subcutaneous haemorrhages) Repeated haemarthroses in th akle, knee, lbow and wrist joints, which have not caused any im paired j int function. Hospitalized few times for haemarthrosis and gastroi testinal he morrhages and has received 2 blood transfusions.

Family 157 Haemophilia A, mild form.



III 3 (O.B.) Born 1899. Excessive haemorrhages fter tooth xtractions d wounds. N haemarthrosis.

III 6 (A.M.) Born 1897 died 1920 of cessive haemorrhage after a tooth traction.

III: 7 (E.J.) Born 1903 Excessive haemorrhages fter tooth extractions d wounds.

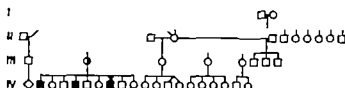
IV 3 (T.T.) Born 1919 Excessive haemorrhages after tooth extractions nd wounds. No haemarthrosis.

IV 12 (S.E.A.) Born 1949 He easily gets haem t mas d subcutaneous haemorrhages after light blows.

V 2 (K.R.) Born 1948. He has bled easily after tooth extraction, t nsillectomy and wounds. Hospitalized for haemorrhage after tonsillectomy and has received 5 blood transfusions. H can attend school normally

V 4 (J.E.R.) Born 1957 First symptom of haemophilia at 7 months of age (excessive haemorrhage after rupture of frenulum linguae) N haemarthrosis. Hospitalized twice for haemorrhages after traumata and has received 2 blood transfusions.

Family 158, Haemophilia A, moderate form.

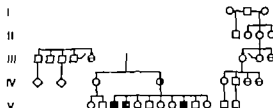


IV 1 (K K) Born 1925 died 1955 of gastrointestinal haemorrhage First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in the knee and elbow joints, which have caused impaired function of the left knee and right elbow joint Hospitalized a few times for haemarthrosis, renal and gastrointestinal haemorrhages and after tooth extractions and had received about 40 blood transfusions. He could attend school normally was free from military service and worked on his father's farm

IV 4 (B K.) Born 1933 died 1936 of gastrointestinal haemorrhage after swallowing a bit of glass. He had repeated subcutaneous haemorrhages.

IV 7 (S h.) Born 1939 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in all the main joints, which have caused impaired function of the knee and right hip joint. Hospitalized a few times for haemarthrosis and renal haemorrhages, and has received about 10 blood and plasma transfusions and one AHF preparation. He could attend school normally and is free from military service. He first aimed to be a watchmaker but is now a clerk.

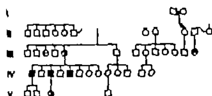
Family 159, Haemophilia A, severe form.



V 4 (A.H) Born 1943 died 1953 of intracranial haemorrhage after a bicycle accident A few haemarthrosis in the ankle and knee joints, which did not cause any impaired joint function Hospitalized a few times for haemorrhages after wounds, and received 3 blood transfusions.

V 9 (R H) Born 1952. First symptom of haemophilia at 9 months of age (subcutaneous haemorrhages) Repeated haemarthrosis in the ankle knee elbow and wrist joints, which has caused impaired function of the ankle and elbow joints. Hospitalized a few times for haemarthrosis, renal and intracranial haemorrhages and haemorrhages after wounds, and has received about 30 blood transfusions. He can attend school normally but is free from gymnastics.

Family 160, Haemophilia A, moderate form.

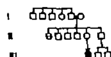


IV:1 (S.E.) Born 1927 First symptom of haemophilia at 7 years of age (haemorrhage after tooth extraction) A few haemarthroses in the ankle and knee joints, which have not impaired the function of the joints. Hospitalized a few times for renal haemorrhage and haemorrhages after wounds and has received about 5 blood transfusions. He could attend school normally has been in military service and works as an engineer

IV 3 (L.E.) Born 1931 First symptom of haemophilia at 6 years of age (excessive haemorrhage after abrasio) No haemarthrosis. Hospitalized a few times for haemorrhages after tooth extractions and gastrointestinal haemorrhage, and has received about 5 blood transfusions. H could attend school normally and has been in military service Works as an agronomist.

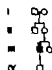
IV 5 (P.E.) Born 1935. First symptom of haemophilia at 6 years of age (haemorrhage after tooth extraction) A few haemarthroses in the ankle joints, which have not impaired the joint function. Hospitalized a few times for haemorrhages after tooth extractions and renal haemorrhage and has received 2 blood transfusions. He could attend school normally has been in military service and is continuing his university studies.

Family 161, Haemophilia A, moderate form.



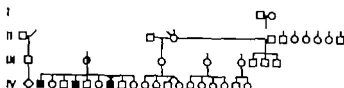
III 1 (B.M.) Born 1938. First symptom of haemophilia at 1 / years of age (haemarthrosis) Repeated haemarthrosis in the ankle knee, hip, elbow and wrist joints, which has caused impaired function of right ankle and knee joints. Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 100 blood transfusions. H could attend school fairly normally is free from military service and works as a watch-maker

Family 162, Haemophilia A, severe form.



III 1 (K.N.) Born 1918. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in the knee shoulder and elbow joints, which have not impaired the joint function. Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 100 blood transfusions. H could attend school normally is free from military service and works as an office manager

Family 158, Haemophilia A, moderate form.

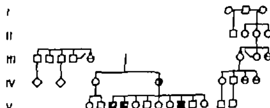


IV 1 (K.K.) Born 1925 died 1955 of gastrointestinal haemorrhage. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses in the knee and elbow joints which have caused impaired function of the left knee and right elbow joint. Hospitalized a few times for haemarthrosis, renal and gastrointestinal haemorrhages and after tooth extractions and had received about 40 blood transfusions. He could attend school normally, was free from military service and worked on his father's farm.

IV 4 (B.K.) Born 1933 died 1950 of gastrointestinal haemorrhage after swallowing a bit of glass. He had repeated subcutaneous haemorrhages.

IV 7 (S.K.) Born 1939. First symptom of haemophilia at one year of age (subcutaneous haemorrhages). Repeated haemarthroses in all the main joints, which have caused impaired function of the knee and right hip joint. Hospitalized a few times for haemarthrosis and renal haemorrhages, and has received about 10 blood and plasma transfusions and one AHF preparation. He could attend school normally and is free from military service. He first aimed to be a watch maker but is now a clerk.

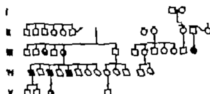
Family 159. Haemophilia A, severe form.



V 4 (A.H.) Born 1943 died 1953 of intracranial haemorrhage after a bicycle accident. A few haemarthrosis in the ankle and knee joint which did not cause any impaired joint function. Hospitalized a few times for haemorrhages after wounds, and received 3 blood transfusions.

V 9 (R.H.) Born 1952. First symptom of haemophilia at 9 months of age (subcutaneous haemorrhages). Repeated haemarthrosis in the ankle, knee, elbow and wrist joints, which has caused impaired function of the ankle and elbow joints. Hospitalized a few times for haemarthrosis, renal and intracranial haemorrhages and haemorrhages after wounds, and has received about 30 blood transfusions. He can attend school normally but is free from gymnastics.

Family 160, Haemophilia A, moderate form.

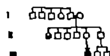


IV 1 (S.E.) Born 1927 First symptom of haemophilia at 7 years of age (haemorrhage after tooth extraction) A few haemarthroses in the ankle and knee joints, which have not impaired the function of the joints. Hospitalized a few times for renal haemorrhage and haemorrhages after wounds and has recei ed about 5 blood transfusions. H could attend school nor mally has been in military ser vice and works as an ngineer

IV 3 (L.E.) Born 1931 First symptom of haemophilia at 6 years of age (excessive haemorrhage after abrasio) No haemarthrosis. Hospitalized a few times for haemorrhages after tooth extractions and gastrointestinal haemorrhage, and has recei ed about 5 blood transfusions. He could attend school normally and has been in military service Works as an agronomist.

IV 5 (P.E.) Born 1935. First symptom f haemophilia at 6 years of age (haemorrhage after tooth extrac tion) A few haemarthroses in the ankle joints, whi h have not impaired the joint function. Hospitalized a few times for haemorrhages after tooth extractions and renal haemorrhage and has received 2 blood transfusions. H could attend school normally has been n military service nd is con tinuing his university studies.

Family 161, Haemophilia A, moderate form.



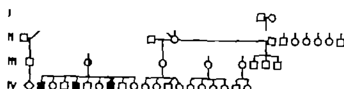
III 1 (B.M.) Born 1938. First ymptom f haemophilia t 1 / years of ge (haemarthrosis) Repeated haemarthrosis in the nkle knee, hip lbow and wrist joints, which has caused impaired func tion of right a kle and knee joints. Hospitalized several times for haemarthrosis and renal haemorrhages, and has recei ed about 100 blood transfusions. He could attend chool fairly normally is free from military service and works a watch-maker

Family 162, Haemophilia A, severe form.



III 1 (K.N.) Born 1918. First symptom of haemophilia at on yea of ge (subcutaneous haemorrhages) Repeated haemarthroses in the knee shoulder and elbow joints, which have not impaired the joint function. Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 100 blood transfusions. He could attend school normally is free from military service and works as office manager

Family 158, Haemophilia A, moderate form.

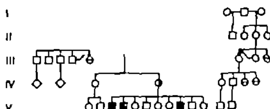


IV 1 (K.K.) Born 1925 died 1955 of *gastrointestinal haemorrhage* First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in the knee and elbow joints, which have caused impaired function of the left knee and right elbow joint. Hospitalized a few times for haemarthrosis, renal and gastrointestinal haemorrhages and after tooth extractions and had received about 40 blood transfusions. He could attend school normally was free from military service and worked on his father's farm.

IV 4 (B.K.) Born 1933 died 1936 of gastrointestinal haemorrhage after swallowing a bit of glass. He had repeated subcutaneous haemorrhages.

IV 7 (S.K.) Born 1939 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in all the main joints, which have caused impaired function of the knee and right hip joint. Hospitalized a few times for haemarthrosis and renal haemorrhages, and has received *about 10 blood and plasma transfusions* and one AHF preparation. He could attend school normally and is free from military service He first aimed to be a watch maker but is now a clerk.

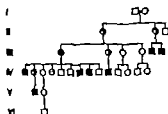
Family 159 Haemophilia A, severe form.



V 4 (A H) Born 1913 died 1953 of intracranial haemorrhage after a bicycle accident A few haemarthrosis in the ankle and knee joints, which did not cause any impaired joint function. Hospitalized *a few times for haemorrhages* after wounds, and received 3 blood transfusions.

V 9 *(R.H) Born 195.. First symptom* of haemophilia at 9 months of age (subcutaneous haemorrhages) Repeated haemarthrosis in the ankle knee elbow and wrist joints, which has caused impaired function of the ankle and elbow joints. Hospitalized a few times for haemarthrosis, renal and intracranial haemorrhages and haemorrhages after wounds, and has received about 30 blood transfusions. *He can attend school normally but is free from gymnastics.*

Family 166, Haemophilia, clinically mild form.



III 5 and 6 Died of excessive haemorrhage at an early age. They bled easily after wounds.

IV:1 (O.J.) Born 1892, died 1922. He bled easily after wounds nd tooth extractions.

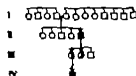
IV:7 (S.J.) Born 1902, died 1929 He had few haemarthroses nd bled easily after tooth extractions.

IV 8 (J.J.) Born 1903, died 1913 He bled easily after wounds and tooth extractions.

IV 10 (E.R.O.) Born 1892. First symptom f haemophilia at 3 years of age (nose-bleedings) Repeated haemarthroses in the ankle knee and elbow joints, which have caused slightly impaired function of the knee joints. Hospitalized a few times for haemorthrosis, renal and gastrointestinal haemorrhages, and has received about 30 blood transfusions. He could attend school normally is free from military service and workes as a tailor

V 1 Born 1920 in U.S.A. and died 3 days after birth of intracranial haemorrhage.

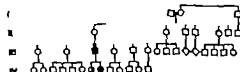
Family 167 Haemophilia B, mild form.



II 5 (O.O.) Born 1903. First symptom of haemophilia t four years of ge (subcutaneous haemorrh ges) A few haem rthroses th knee joi ts, wh i h have n t caused any impaired joint function. Hospitalized several times for haematoma in th muscles in combination with renal and gastrointestinal haemorrhages. He has worked as a lumberman, but is now disabled and lives on pension

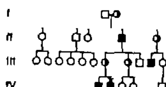
IV 1 (H.K.) Born 1956. First symptom of haemophilia at two years of ag (subcutaneous haemorrhages) No haemarthrosis. Hospitalized once for haemorrhages after tooth extraction and received one blood transfusion. He can attend ordinary nursery school.

Family 168, Haemophilia A, severe form.



III 3 (L.S.) Born 1927 First symptom of haemophilia t on year of age (subcutaneous haemorrhages) Repeated haemarthroses in the ankle knee elbow and wrist joints, which ha caused impaired function f both knees a d the right lbow joint. Hospitalized several times for haemarthrosis, gastrointestinal and renal haemorrhages a d has received about 40 blood transfusions. He could attend school f irly normally is free from military service and is a textile worker

Family 163, Haemophilia B, mild form.



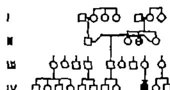
II 4 (O O) Born 1895 He has always bled easily after tooth extractions and wounds. No haemarthrosis.

III 10 (H H) Born 1928 First symptom of haemophilia at 10 years of age (tooth extractions) He has bled from the nose easily has had haemarthrosis in the knee joint once but never hospitalized and has not received any blood trans fusions. He could attend school normally has been in military service and works as an electri cian.

IV 1 (B.O) Born 1943 First symptom of haemophilia at 2 years of age (nose-bleedings) A few times haemarthroses in the knee joints which have not caused any im paired joint function Hospitalized a few times for haemorrhages af ter minor surgery and has received about 15 blood transfusions. He can attend school nor mally

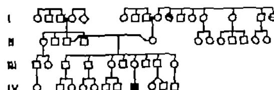
IV 2 (T O) Born 1947 First symptom of haemophilia at 2½ years of age (nose bleedings) No haemarthrosis. Hospitalized a few times for haemorrhages after wounds and renal haemorrhage, and has received 5 blood transfusions. He can attend school normally

Family 164, Haemophilia A, severe form.



IV 9 (B.E.O) Born 1945 First symptom of haemophilia at 9 months of age (subcutaneous haemorrhages) Repeated haemarthrosis in all the main joints, which has caused severely impaired function of the knees and the right elbow joint. Hospitalized several times for haemarthrosis, renal and gastrointes tinal haemorrhages, and has received about 70 blood and plas ma transfusions. He can attend or dinary school, but is away for long periods of time.

Family 165, Haemophilia A, moderate form.



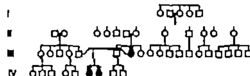
IV 8 (E.O) Born 1947 First symptom of haemophilia at 1 / years of age (excessive haemorrhage after a wound in the mouth) A few haemarthroses in the ankle knee and hip joints, which have not caused any impaired joint function. Hospitalized a few times for haemarthrosis, and has received about 5 blood transfusions. He can attend school normally

III: 5 (B.) Born 1918, died 1923 of haemophilia. He alw ys bled easily after wounds.

III 6 (B.) Born 1921 died 1923 of haemophilia. Bled easily after wounds.

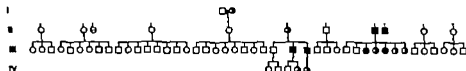
IV 5 (P.O.H.) Born 1932, died 1957 f interior haemorrhage. First symptom of haemophilia at 10 months of age (excessi e haemorrhages from a wound in the mouth). Repeated haemarthrosis in the ankle and elbow joints, which caused impaired functi n of the right elbow. Hospitali ed several times for haemarthrosis, haematoma in the muscles and excessive haem rrh ges from wounds, and received about 20 blood transfusions.

Family 173, Haemophilia B, moderate form.



IV: 7 (L.B.) Girl, born 1954. First symptom of haemophilia at on year of age (subcutaneous haemorrhages). Repeated haemarthroses in all the main joints, which have not yet caused impaired function of joints. Hospitalized a few times for haemorrhages afte wounds d nose bleeding, and has received on blood transfusion. The case will be published separately.

Family 174, Haemophilia A, mild form.



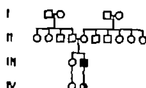
II 8 (D.K.) Born 1906 d ed 1960 of postoperative haemorrhage after laparotomy. H had tendency to bleed fter tooth e tracti ns and m or surgery.

II 9 (H.K.) Born 1908, died of unknown cause at an early ge. H alw ys bled easily from wounds, but had haemarthrosis.

III 27 (B.S.) Born 1928. First symptom of haemophilia t 7 years of ge (excessive haemorrhage after a minor operation). No haemarthrosis. Hospit lized a few times for nose-bleedings and haemorrhages after tooth extractions, and has received 3 blood transfusions. He could attend school normally, has been in military service, and works as a ma hine repairman.

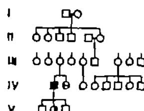
III 28 (D.S.) Born 1935. First symptom of haemophili t 20 years f ge (haemorrhages after tooth extractions). N haemarthrosis. Never hospitalized.

Family 169, Haemophilia A, moderate form.



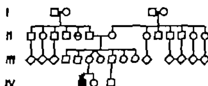
III 2 (B.S.) Born 1909 First symptom of haemophilia at 2 years of age (haemarthrosis) Repeated haemarthroses in all the main joints, which have caused severely impaired function of the knee and hip joints. Can walk with difficulty Hospitalized several times for haemarthrosis, renal and gastrointestinal haemorrhages, haemorrhages after tooth extractions, and has received about 20 blood transfusions. He could attend ordinary school but was away long periods of time Is free from military service formerly worked as a lift operator but has now disability pension

Family 170, Haemophilia, clinically mild form.



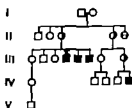
IV 1 (L.W.) Born 1913 First symptom of haemophilia at 12 years of age (excessive haemorrhage after appendectomy) No haemarthrosis. Hospitalized a few times for excessive haemorrhages after laparatomy tooth extractions, one renal haemorrhage, and a few haematomas in the muscles, and has received about 5 blood transfusions. He could attend school normally Is free from military service and works as an accountant

Family 171, Haemophilia B, moderate form.



IV 1 (T.Y.) Born 1941 First symptom of haemophilia at 2 years of age (subcutaneous haemorrhages) Repeated haemarthroses in the knee joints, which have not caused any impaired joint function. Hospitalized several times for haemarthrosis and haemorrhages after tooth extractions and has received about 15 blood transfusions. He could attend school normally and works as an office apprentice.

Family 172, Haemophilia A, severe form.



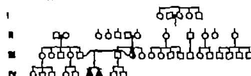
III 4 (J.B.) Born 1908. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in the elbow joints, which have caused severely impaired function of the right elbow joint (he has had polio and has partial paralysis of the left leg) Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 10 blood transfusions. He could attend school normally and works as a tailor

III 5 (B.) Born 1918, died 1923 of haemophilia. He always bled easily after wounds.

III 6 (B.) Bo n 1921 died 1923 of haemophilia. Bled easily after wounds.

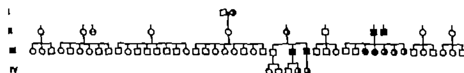
IV 5 (P.O.I.L.) Born 1952, died 1957 of interior haemorrhage First symptom of haemophilia t 10 months of age (excessive haemorrhages from a wound in the mouth) Repeated haemarthrosis in the ankle and elbow joints, which caused impaired fun tion of the right elbow Hospitalized several times for haemarthrosis, haematoma in the muscles and excessive haemorrhages from wounds, nd received about 20 blood transfusions.

Family 173, Haemophilia B, moderate form.



IV 7 (L.B.) Girl, born 1954. First symptom of haemophilia at one year of ge (subcutaneous haemorrhages) Repeated haemarthroses in all the main joints, which ha e not y t caused impaired function of j ints. Hospitalized few times for haemorrhages aft r wounds and nose bleedi g, and has received ne blood transfusion. Th case will be published separately

Family 174, Haemophilia A, mild form.



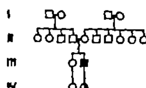
II 8 (D.K.) Born 1904, died 1960 of post operati haemorrhage after laparatomy. H had tendency to bleed fter tooth xtracti ns and minor surgery

II 9 (H.K.) Born 1902, d ed of unknown cause t an early ge. He lw y bled easily from wounds, but had no haemarthrosis.

III 27 (B.S.) Born 1928. First symptom of haemophilia at 7 years of age (excessive haemorrhage after minor operation) N haemarthrosis. Hospitalized a few times for nose-bleedings and haemorrhages after tooth extractions, and has received 3 blood transfusions. He could ttend school normally has been in military service, and works as machine repairman.

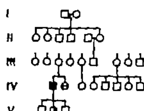
III 28 (D.S.) Born 1935. First symptom of haemophilia at 20 years of age (haemorrhages fter tooth extra tions) No haemarthrosis. Never hospitalized.

Family 169 Haemophilia A, moderate form.



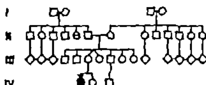
III 2 (B S.) Born 1909 First symptom of haemophilia at 2 years of age (haemarthrosis) Repeated haemarthroses in all the main joints, which have caused severely impaired function of the knee and hip joints. Can walk with difficulty Hospitalized several times for haemarthrosis, renal and gastrointestinal haemorrhages, haemorrhages after tooth extractions, and has received about 20 blood transfusions. He could attend ordinary school but was away long periods of time is free from military service formerly worked as a lift operator but has now disability pension

Family 170, Haemophilia, clinically mild form.



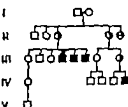
IV 1 (L.W) Born 1913 First symptom of haemophilia at 12 years of age (excessive haemorrhage after appendectomy) No haemarthrosis. Hospitalized a few times for excessive haemorrhages after laparatomy tooth extractions, one renal haemorrhage and a few haematomas in the muscles, and has received about 5 blood transfusions. He could attend school normally is free from military service and works as an accountant.

Family 171, Haemophilia B, moderate form.



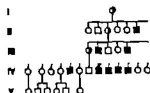
IV 1 (T N) Born 1911 First symptom of haemophilia at 2 years of age (subcutaneous haemorrhages) Repeated haemarthroses in the knee joints, which have not caused any impaired joint function. Hospitalized several times for haemarthrosis and haemorrhages after tooth extractions and has received about 15 blood transfusions. He could attend school normally and works as an office apprentice

Family 172, Haemophilia A, severe form.



III 1 (J B) Born 1908. First symptom of haemophilia at one year of age (subcutaneous haemorrhages) Repeated haemarthroses in the elbow joints, which have caused severely impaired function of the right elbow joint (he has had polio, and has partial paralysis of the left leg) Hospitalized several times for haemarthrosis and renal haemorrhages, and has received about 10 blood transfusions. He could attend school normally and works as a tailor

Family 178, Haemophilia A, moderate form.



The family is f Finnish origin and described by Ikkala under number A 1 5. An abstra t of the pedigree will be given.

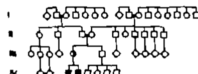
IV 8 (M.L.) (Ikkala A 1 5, IV-8) Born 1938. First ymptom of haemophi lia t 2 years of ge (ex essive haemorrhage from a wound in one finger) Repeated haemar throses in th nkle knee elbow and wrist joints, which ha caused slight impaired function of the lbow joints. Hospitalized once for haemorrhages after tooth ex tractions. He could attend school normally is free from military service and wo ks as a kitchen att ndant.

Family 179, Haemophilia A, severe form.



IV 10 (G.L.) Born 1945 First symptom of haemophilia t 2 years of age (subcutaneous haemorrhages) Repeated haemarthroses in the ankle, knee lbow nd wrist joints, wh h have not caused a y impa red joint function. Hospitalized several times for haemar throsi and once for renal haemorrhage, and has received about 35 blood tra sfusions. He can at t d ordinary school, but is free from gymnastics.

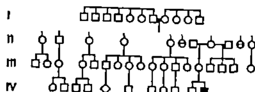
Family 180, Haemophilia B, moderate form.



IV 1 (L.O.K.) Born 1939. First symptom f haemophilia t ne year of age (ubcutaneous haemorrhage) Repeated haemarthroses i all the mai joi ts, which have caused impa red f ction of the elbow joi t Hospitalized several times for haemarthrosis, renal haemorrhages and haemorrhages af ter tooth extracti ns, nd has received bout 15 blood transfusions. H could ttend school nor mally is now at vocational trainl g school and aims to be a TV technician. He is free from mili tary service

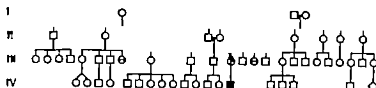
IV 2 (G.K.) Born 1942. First symptom of haemophilia t one year of age (subcutaneous haemorrhages) Re peated haemarthroses in all the mai joints, which have caused impaired function of the knees and the right elbow joi ts. Hospi talized several times for haemar throsis, renal a d gastrointestinal haemorrhages, and has received about 20 blood transfusio s. He could attend school fairly nor mally d is now t a vocation l training school in order to be a TV-tech ician.

Family 175, Haemophilia A, severe form.



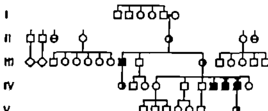
IV 10 (L.W.) Born 1950 First symptom of haemophilia at 6 months of age (excessive haemorrhage after dentition) Repeated haemarthroses in the ankle and knee joints, which have not caused any impaired joint function. Hospitalized a few times for haemarthrosis and haemorrhages after wounds and has received ? blood transfusions.

Family 176, Haemophilia B, severe form.



IV 14 (L.G.L.) Born 1959 First symptom of haemophilia at one year of age (subcutaneous haemorrhages) No haemarthrosis yet. Hospitalized once for haematoma.

Family 177 Haemophilia B, mild form.



III 7 (K.O.) Born 1891 died 1956 of haemorrhage after an accident. He always bled easily after tooth extractions and wounds.

IV 7 (B.H.) Born 1930 First symptom of haemophilia at 5 years of age (dentition) He has had a few haemarthroses in the ankle joints, which have not impaired the joint function Excessive haemorrhages after tooth extractions, but he has never been hospitalized or received any blood transfusions. He could attend school normally and has been in military service and works as a controller in a factory

IV 8 (S.O.H.) Born 1935 First symptom of haemophilia at 16 years of age (gastrointestinal haemorrhage) No haemarthrosis. Hospitalized several times for gastrointestinal haemorrhages and once for haemorrhage after tooth extraction bled excessively after gastric operation and has received about 80 blood transfusions. He could attend school normally is free from military service and works as a controller in a factory

IV 9 (A.H.) Born 1938. First symptom of haemophilia at 1– years of age (excessive haemorrhage after tonsillectomy) A few haemarthroses in the ankle and knee joints, which have not caused any impaired joint function. Hospitalized once for haemorrhages after tonsillectomy and has received about 5 blood transfusions. He could attend school normally is free from military service and works as a foreman in a factory

carriers were examined Low AHF values, ranging from 15 to 60 per cent of normal were found in 31 definite, 9 probable and 16 potential carriers. Of the 35 definite and probable carriers of fertile age, 34 had consistently low AHF values. The definite carrier in family *114* had a normal AHF value on four occasions and a low value on one occasion Five definite or probable carriers over the menopause had normal AHF values. However in a group of normal healthy women over the menopause, the mean plasma AHF content was found to be significantly higher than in a group of normal women of fertile age This might explain why it was not possible to demonstrate low AHF values in all the carriers over the menopause

From the genetic point of view the potential carriers in this series of *female relatives of haemophiliacs* could be expected to contain about 50 per cent carriers and 50 per cent normal individuals. The low AHF content in 16 of the 32 potential carriers investigated is in accordance with this view

The results of the investigation of carriers of haemophilia A provided evidence that such carriers of fertile age can be traced by demonstration of low AHF values. Consistently low AHF values on repeated occasions are a strong argument in favour of the woman in question being a carrier If on the contrary normal values are found on repeated occasions, the woman in question is, in all probability not a carrier

In *haemophilia B* families 41 women were investigated 18 of them were definite or probable and 18 potential carriers. Low B factor values, ranging from 15 to 60 per cent of normal, were found in 15 of the 18 definite all 5 probable and 7 of the 18 potential carriers.

With the recalcification method used for assay of the B factor it seems possible to state that demonstration of a low value in a woman belonging to a family with haemophilia B does in fact, mean that she is a carrier If normal values are obtained, it is most likely that the woman is normal although the possibility that she is a carrier cannot be completely ruled out

III Symptomatology of haemophilia A and B

The bleeding manifestations their incidence and sequelae, were studied in the 176 patients classified as having severe, moderate or mild haemophilia A or B, according to the plasma content of AHF or B factor respectively An account of the results is given in Paper III It was found that as far as the severity of the bleeding manifestations is concerned no difference was present between haemophilia A and B with a corresponding level of the coagulation factors in question.

In the three forms of haemophilia classified according to the AHF or B factor level, respectively the clinical features can be summarized as follows, on the basis of the symptomatology

Severe haemophilia (AHF or B factor <1 per cent of normal) The symptoms generally appear during the first two years of life, and the diagnosis is usually obvious at the first visit to hospital. Repeated haemarthroses occur in ankle knee elbow and wrist joints at an early age, and result in impaired joint function

Results and Comments

I *Coagulation studies of haemophiliacs*

Coagulation studies were made of 176 haemophilic patients belonging to 130 Swedish families the results are given in Paper I Totally 101 families were found to have haemophilia A and 20 haemophilia B i e., an incidence of 78 per cent of haemophilia A and 22 per cent of haemophilia B, thus in good agreement with the figures reported in other countries (Paper I table I)

In the families with haemophilia A 58 families (71 cases) were found to have severe haemophilia 18 (28 cases) moderate and 25 (34 cases) mild haemophilia In the families with haemophilia B 12 families (19 cases) had severe 8 (10 cases) moderate and 9 (14 cases) mild haemophilia The Swedish series thus consisted of about the same number of severe haemophiliacs as of moderate and mild collectively

The affected members of the individual family had the same type of haemophilia as well as the same AHF and B factor level No combined coagulation defects were found in the Swedish series with the exception of one family with moderate haemophilia B (family 150) in which the AHF values were on the low side of normal and one case (family 107) of mild haemophilia A with factor V deficiency This is in contrast to the findings of Sjolin (14) whose series contained 18 families with combined deficiency of AHF and B factor A possible explanation is the test methods used by him so that some of his combined defects might in fact have been only one defect combined with an anticoagulant

The Swedish patients with severe and moderate haemophilia were found to have a prolonged coagulation time and a pathological value for prothrombin consumption Of the mild haemophiliacs 50 per cent had a normal coagulation time and about 30 per cent had normal prothrombin consumption Normal values were recorded for factor V (except in the aforementioned family 107) prothrombin + proconvertin bleeding time and platelet count In both haemophilia A and B, the mean values for fibrinogen were above the normal range Circulating anticoagulants were found to be present in 8 patients.

II *Coagulation studies of carriers*

Coagulation studies were made of 79 definite probable or potential carriers of haemophilia A and 41 definite, probable or potential carriers of haemophilia B The results are reported in Paper II

A *definite carrier* was defined as

a. A woman who is a daughter of a haemophiliac

b A mother with two or more children with haemophilia and/or proved carrier state

c A mother of a single haemophiliac or of a proved carrier offspring and with other haemophilic relatives

A *probable carrier* was defined as a woman with only one haemophilic son or one haemophilic daughter's son but no other relatives with haemophilia

A *potential carrier* was defined as a woman without haemophilic sons but with a 50 to 25 per cent genetic chance of being a carrier of haemophilia

In families with *haemophilia A* 33 definite, 14 probable and 32 potential

The geographical origin of the heredity line was traced for about four generations, namely to around 1850 In the families with a definite heredity line, i. e having a haemophiliac or a carrier born in 1850 or earlier the birthplace of this member was taken as the geographical origin of the family When there was no definite heredity line — these families being denoted as having a "possible heredity — a postulated heredity line was traced on the female side from the haemophilic member The geographical origin of the families was evenly distributed over the country in relation to the population density with the exception of 8 families with severe or moderate haemophilia B, all originating from a limited region in the south-east of Sweden. A possible explanation is consanguinity between some of these families in earlier generations.

Totally 26 of the 180 families had no living haemophiliac. Only four families had, as far as could be ascertained, no potential carrier of fertile age. It can be calculated from the pedigrees that there are at least 75 carriers f haemophilia A and B in Sweden born in 1930 or later The corresponding number of carriers of moderat and mild haemophilia is 29 and 34 respectiv ly Consequently it can be expected that both haemophiliacs and carriers will be born in these families during the next few decades.

Tw cases f haemophilia in women w re detected, ne of severe haemophilia A (family 32) in girl with male sex hromatin pattern, and one of moderat haemophilia B (family 173) in a girl with no signs of sexual anomalies.

\ *Medico-social aspects of haemophilia*

The *incidence of haemophilia* in Sweden, both live births and living haemophiliacs, was estimated in Paper \ As pointed out previously only half of the patients with mild haemophilia had a prolonged clotting time in whole blood. Their bleeding manifestations were sporadic and usually of mild degree and caused no disablement Moreover moderate or mild haemophilia is not always detected at an early age. It is therefore highly probable that the real number of mild haemophiliacs born during a giv n period was higher than that found in the present series This implies that the number born in the last period must be corrected for the late appearance of the disease. With this correction and correction for the undetected cases of mild haemophilia the estimated incidence of living haemophiliacs (all forms) is one living haemophiliac per 11,000 men and one haemophiliac born per 8,000 liveborn males

The *mild cases of haemophilia* constitute a special clinical and medicosocial problem not comparable with that in severe or moderate haemophilia. In the present series, bleeding manifestations generally appeared only in connexion with major or minor surgery injuries or gastrointestinal ulcers haemophilia was, *in fact* ften diagnosed for the first time on such an occasion. Bleeding was then as a rule as profuse and dangerous as in the severe form f haemophilia.

The mild forms of haemophilia had, however only slight medico-social consequences. Since the unrecognized mild forms required neither hospitalization nor ambulant therapy

after the age of 10 Moreover severe changes in the joints often give rise to moderate or severe disablement Large subcutaneous intramuscular and retroperitoneal haematomas appear spontaneously or after slight trauma Episodes of haemorrhage from the renal and/or gastrointestinal tract are common They require frequent hospitalization and often regular transfusions of blood or plasma The coagulation time is appreciably prolonged *i e.*, to more than 30 minutes, whereas the bleeding time is normal

Moderate haemophilia (AHF or B factor 1—4 per cent of normal) The first symptoms generally appear before 8 years of age Haemarthroses are less frequent than in severe haemophilia and as a rule are restricted to a few joints, usually ankle knee or elbow They do not lead to impaired function of the joints before middle age Spontaneous haematomas in the subcutis muscles and retroperitoneum are rare Sporadic episodes of renal or gastrointestinal haemorrhage occur but require hospitalization only when the bleeding is profuse. Blood or plasma transfusions are usually required in the presence of haemarthrosis and renal or gastrointestinal haemorrhage The coagulation time is prolonged ranging from 10 to 45 minutes

Mild haemophilia (AHF or B factor 5—25 per cent of normal) In half of the cases the initial symptoms appear before 8 years of age and in one-fourth of them in adolescence Haemarthrosis occurs in not more than half of the cases and only after moderate or severe trauma It is restricted to one or two joints, and does not lead to impaired joint function The bleeding episodes take place after tooth extraction or in connexion with minor and major surgery or only as repeated renal or gastrointestinal haemorrhage. Hospitalization is rare and blood transfusions are needed only when bleeding is profuse The coagulation time may be prolonged, but is within the normal range in half of the cases

IV Hereditary investigations

Of the 180 haemophilic families investigated 173 could be followed for three or more generations the results are described in Paper IV A positive heredity — by which is meant that the haemophilic gene could be traced in at least two generations — was demonstrated in 127 of these families, *i e* in 73 per cent This high incidence of positive heredity is partly explained by the conclusions that could be drawn from the carrier investigations (Paper II) and the previous investigation by Sköld (15) It was possible to trace haemophilic members of 8 "new" families in his unpublished material and thus to demonstrate a positive heredity despite the fact that the living haemophilic members were completely unaware of a family history of the disease

In 39 families, two or more haemophiliacs were tested all the affected members of the individual family were found to have the same type of haemophilia A combined deficiency of B factor and a slightly low AHF level was recorded in all 5 tested members of family 150 (two haemophiliacs and three definite carriers)

In family 10, a low factor V level was found in the only haemophiliac tested

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Totally 20 of the 180 families had no living haemophiliac Only four families had as far as could be ascer tained, a potential carrier of fertile age. It can be calculated from the pedigrees that there are at least 75 carriers f haemophilia A and B in Sweden, born in 1930 or later The corresponding number of carriers of moderate nd mild haem philia is 29 and 4 respectively Consequently it can be expected that both haemophi liacs and carriers will be born in these families during the next few decades.

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In family 107 a low factor V level was found in the only haemophiliac tested

mean values for moderate and mild haemophiliacs were 0 7 and 0.3 week s hospitalization and 1.0 and 1.2 blood transfusions, respectively The young er haemophiliacs had more admissions to hospital per year but a short er average duration of hospitalization than the older haemophiliacs

Haemophilia especially the severe form, gives rise to many *medico-social problems* The following scheme is suggested for the medico-social management of haemophilia.

The early establishment of a correct *diagnosis* based on a thorough study of the coagulation system including determination of the plasma content of the factor in which the pa tient is deficient is essential both for proper clinical management, and for the patient s prospects of living a normal life. The patient should be provided with an *identity card* giv ing the basic information such as the type f haemophilia, blood group, th essential for emergency therapy and addresses of doctors who know the patient

The patient his parents and/or family must be given full information about the disease about the impor tanc f *prophyla tic d ntal care* and the treatment of minor *bleeding episodes* He should be given *educational* nd *vocational training* fo a suitable occupation that does not exaggerate or provoke bleeding episodes. The *family history* should be penetrated, in rder to trace all potential carriers, who h uld be in estigated to disclose the rrier state The *d finite carriers* mu t be nformed of the risk of giv ing birth t new haemophiliacs or carriers and, if pregnant, must be granted *permissi n fo abortion*

VI *Treatment of haemophilia A with the AHF preparation*

Fraction I-0 (3) containing most of the AHF content of plasma was first tested in the treatment of haemophilia A in 1956 and has been available for clinical purposes since 1958 An account of the therapeutic results achieved with this preparation i given in Paper VI

Totally 63 patients with haemophilia A were treated. They were given altogether 497 whole doses of the AHF preparation (one whole dose prepared from 1400—1600 ml of plasma) in 818 administrations (injections) for 277 bleeding episodes.

Major surgery e g appendectomy cholecystectomy laparotomy for ileus, nephrolithiasis and nephrectomy explorative laparotomy was perform ed in 6 patients (8 episodes) They were given 159 administrations (116.5 whole doses) of the AHF preparation *Minor surgery e g* dental extraction, joint operations, incision of phleg mon was done in 12 patients (19 episodes) These patients were given 144 admini trations (92 whole doses)

Bleedings in the joints or muscles, retroperitoneal, gastrointestinal and intracranial haemorrhage haematuria and postoperative bleeding were also treated Here, the *total* was 165 epi sodes and 430 administrations (245 whole doses)

Prophylactic treatment was given to three patients with severe haemophilia A. They received fraction I-0 85 times (43.5 whole doses)

The results were discussed in de tail in Paper VI. Our experience could be summarized as follows.

After injection of fraction I-0, abnormal bleeding was arrested, and surgical procedures could be perform-

they can be disregarded from the medico-social point of view

From the medico-social point of view the care of *haemophiliacs* can be divided into three periods

Period I 1900—1942
Period II 1943—1957
Period III 1958 onwards.

Before 1943 no special arrangements had been made for treatment or training of the haemophiliacs Period II was initiated in 1943 by the work of E. Sköld. He organized blood *transfusion centres, and made* arrangements for special vocational training as well as for giving information to haemophiliacs and carriers regarding birth-control and sterilization on eugenic grounds. Since 1958, fresh plasma and new plasma derivatives *including* concentrated *AHF* have been available for therapeutic purposes. In view of the short time that has elapsed the results of this therapy cannot, however yet be evaluated *from* the medico-social aspect

On the other hand the results in period II and in period I can be evaluated by comparing the *causes of death* and *mean age at death* in these two periods It was found that the main causes of death had changed from *haemorrhage after* wounds and haemorrhage of unspecified localization in the earlier period to cerebral and *gas*trointestinal haemorrhage in the later period The mean age at death for severe haemophiliacs was 16.5 years in the earlier period and was pro*longed to* 23.2 years during the later one The corresponding ages for moderate *haemophiliacs* were 19 9 *and* 40 1 years and for mild haemophiliacs 29 6 and 50 0 years respectively

At the time of writing the average life expectancy for severe haemophiliacs could therefore be estimated to be one-third that of normal male inhabitants of Sweden, the corresponding figures for moderate and mild haemophiliacs being one-half and two-thirds, respectively

A *social review* was *compiled from* the data *supplied in answer to a questionnaire* by 235 haemophiliacs, and from hospital records Adequate *education* had been obtained by 60 per cent of the severe haemophiliacs one-third of them in a special boarding school *for the handicapped, or in the* home Of the moderate haemophiliacs, 77 per cent had received adequate education Of those over 20 years of age, altogether about 60 per cent of the severe and moderate haemophiliacs had received *vocational training*

All but one of the 20 severe and moderate haemophiliacs doing intellectual or light work could earn their living Only one-fourth of the severe and half of the moderate haemophiliacs doing light manual work were *self-supporting No severe and only* one moderate haemophiliac could perform heavy manual work

Of 45 severe haemophiliacs over 20 years of age, 12 (27 per cent) had no occupation and lived solely on a *disablement pension* Another 15 severe *haemophiliacs* had a disablement pension but worked sporadically The corresponding figures *for 31 moderate* haemophiliacs were 2 with a disablement pension and no occupation and 7 with a disablement pension but doing sporadic work

During 1943—1957 the severe haemophiliacs had a mean *hospitalization time* of one week per haemophiliac and year and received an average 3 9 *blood* transfusions. *The corresponding*

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Th patient, his parents and or fa mily mu t be given full information about the disease about the impor t nce of *prophyl tic dental care* and the treatment of min r *bleeding epi sod* He should be given *educational* and *vocational training* for a suitable occupation that does not exaggerate or provoke bleeding episodes. The *fa m ly history* should be penetrated in order t trace all potential *carriers*, who h uld be investigated to disclose the carrier state The *definite carriers* must be informed of the risk of giv ing birth t new haemophiliacs or carriers and if pregnant, must be granted *permission for abortion*

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The results were discussed in de tail in Paper VI Our experience could be summarized as follows.

After injection of fraction I-0 abnormal bleeding was arrested and *surgical* procedures could be perform

Table II Blood groups in Swedish haemophiliacs

Blood group: System	Group	Type	No. of haemophiliacs	Immunized: Type of antibody	No. of cases
ABO	O		55		
	A		40		
	A		11		
	B		10		
	A B		5		
	A_2B		1		
Rh	Rh+	Rh rh (C+ E— c+ e+)	50	anti—E	1
		Rh_1Rh (C+ E— c— e+)	19	anti—c	1
		Rh_2rh (C— E+ c+ e+)	15		
		Rh_2Rh_2 (C— E+ c+ e—)	4		
		Rh Rh_2 (C+ E+ c+ e+)	12		
		Rh Rh (C+ E+ c— e+)	1		
	Rh—	rhrh (C— E— c+ e+)	21	anti—D	4
				anti-(C D)	3
MN	M		28		
	N		21		
	MN		73		
Kell	K+	Kk and KK	15		
	K—	kk	107	anti—K	2
Duffy	Fya+		55		
	Fya—		67		
Total no. of haemophiliacs			122		11

ed without any haemorrhagic complications, provided that the plasma AHF was kept at a sufficiently high level In order to control severe bleeding episodes, it was necessary to raise the AHF level of the patient to 30—50 per cent of normal immediately and then to maintain it at about 20—30 per cent until healing was complete In connexion with surgery the plasma AHF level was kept at 30—70 per cent during operation and at about 20—40 per cent in the postoperative course For management of haemorrhage, such as joint bleeding, haematoma and haematuria an AHF level of 10—20 per cent appeared sufficient The advantages of therapy with fraction I-0 in haemarthrosis and spontaneous haematoma are difficult to evaluate

Prophylactic treatment with half a dose of fraction I-0 once a month, was given to 3 boys with severe haemophilia A for 2 to 3 1/2 years During this period the frequency and severi

ty of bleeding episodes diminished and, judging by the plasma AHF level and the clinical features, it seemed as if the severe form had changed to a moderate form.

No side effects were observed after injection of fraction I-0. No resistance or diminished clinical response to the preparation was demonstrated in the patients, and no circulating anticoagulants appeared; this applied even to those who had received the largest number of infusions. This is in contrast to blood or plasma therapy when immunization against blood-group antigens is common.

In connexion with blood-group determinations for the issue of identity cards to haemophiliacs, their serum was investigated for immune blood group antibodies. The results of these determinations are given in table II. Eleven cases of immunization against blood-group antibodies were detected. Two other haemophiliacs had agglutinating antibodies against leukocytes, when investigated on different occasions.

Up to now no differentiation has been made between haemophilia A and B as far as their clinical features and medico-social effects are concerned. However, in view of the better possibilities of treating haemophilia A patients with the AHF preparation, it seems likely that these two types may have to be evaluated separately in the future.

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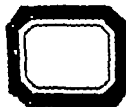
ACTA MEDICA SCANDINAVICA

Supplementum 378

Kidney Function Studies with ^{131}I-Tagged Sodium Ortho-Iodohippurate

By

GÖSTA MAGNUSSON



Accompanies Vol. 171

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The chief editors have been. Axel Key 1869—1900 C. G Santesson 1901—1915 I *Holmgren 1916—1957 and Birger Strandell 1958* to date

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From the Department of Medicine (Head Prof. G Biörck)
and
the Department of Clinical Chemistry (Head: Assistant Prof. H. Boström)
Karolinska Institutet, Serafimerlasarettet, Stockholm, Sweden.

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Preface

I am greatly indebted to my chief, Professor Gunnar Biörck for valuable advice and constructive criticism of the manuscript.

I am sincerely grateful to Assistant Professor Harry Boström for placing the laboratory facilities of the Department of Clinical Chemistry at my disposal and for help with the electrophoretic examinations.

To the former chief for the Department of Clinical Chemistry Assistant Professor Sven Gardell, I am much obliged for his valuable advice on the chromatographical investigations.

I wish to thank my friends and collaborators Doctors Leo Meurman and Gunnar Carlberger who have shown a never failing and stimulating interest for the present work and in all respects given generous help without which the investigation would not have been possible.

I want to acknowledge my great indebtedness to Assistant Professor Erik Odeblad at the Isotope Laboratory of the Department of Obstetrics and Gynecology, Sabbatsberg Hospital, for awakening my interest in radioisotope technique in medical research, which prepared the approach to the present investigation.

To Doctor Rune Ordell I wish to express my thanks for his advice and useful discussions.

I would like to thank Docent Harald Elmsch for his kind interest in and valuable criticism of the clinical part of the present investigation.

I have been fortunate in being able to discuss chemical problems with Assistant Professor Lars Melander and Doctor Bo Lamm at the Nobel Institute of Chemistry Stockholm. For the great advantages thereby implied I am most thankful.

For valuable pharmaceutical information I thank very much Mrs Anna Lisa Holge, pharmaceutical chemist at Serafimerlasarettet.

To Mrs Maria Déry I wish to express my thanks for technical help for performance of the electrophoretical investigations.

I am also greatly indepted to Mrs Gunborg Rachow and Mrs Birgit Hambring for good secretarial assistance.

The investigations have been supported by grants from Karolinska Institutet and Swedish National Association against Heart and Chest Diseases.

During part of the investigation, the author held a doctoral fellowship at Karolinska Institutet.

Stockholm, April 1962.

Gösta Magnusson

CHAPTER I

Survey

Introduction

The kidney is the dynamic center with the help of which the composition and amount of urine can be varied in order to maintain a homeostatic equilibrium in the body which means the appropriate level and proportion of the electrolytes in the circulation and extracellular space as well as in the living cells will be maintained. Further the relation of acid to base can be regulated as well as the metabolism of e.g. glucose amino acids, vitamins, and metabolic products. The kidney has also the important power to excrete body foreign substances among which several drugs can be mentioned. As early as in the beginning of this century it was found that this last mentioned quality of the kidney could be used to test its function with the help of certain nephrotropic dye substances. This was performed by injecting into the blood the dye compound in question and subsequently following its rate of excretion in the urine. This principle was applied by Rowntree and Geraghty in 1910, when they introduced phenol red into the study of kidney function. This method constituted the first trial of estimating quantitatively the kidney function.

Another attempt to evaluate the kidney function quantitatively was made by Ambard and Weill in 1912, who administered urea and related its excretion rate in the urine to the content of urea in the blood.

Much later in 1929 Möller *et al.* introduced the term *clearance* in clinical measurement of the urea excretion. By urea clearance they meant the amount of blood completely cleared from the test agent per time unit. During the 1930's this expression was applied to the excretion by the kidney of a number of agents, among which the most important were inulin (Richards *et al.*, 1934) and some substances used as contrast media in excretory urography e.g. Diodrast® Skiodan® and Hippuran® (Elsom *et al.*, 1934—35 Elsom *et al.*, 1936, Elsom *et al.*, 1937). Owing to the almost complete renal extraction of these last mentioned agents, their clearance values represented also the renal blood flow. The importance of such determinations in the clinical practice was pointed out by Smith *et al.* in 1938. However in cases with kidney disease the renal extraction power is diminished, and the clearance values do not represent the blood flow through the kidneys. This error could be allowed for when Warren *et al.*, in 1944 introduced the technique of renal vein catheterization. Another parameter of importance in the study of kidney function, i.e. the maximal tubular excretion

Magnusson, 1960, Whitley et al. 1960, and zum Winkel and de Maria, 1961). Kidney function studies have been described in a great number of investigations during 1956—1962, in which the test has been performed with various ^{131}I labelled substances, e.g. the diethanolamine salt of 3,5 diiodo-4-pyridone-N-acetic acid, Diodrast® Diodon® (Taplin et al., 1956, and others) sodium 3-acetamide 2,4,6-triiodobenzoate Urokon Triopac® (Taplin et al. 1956, Winter and Taplin, 1958, and others) the methylglucamine or sodium salt of 3,5-diacetamide-2,4 6-triiodobenzoate, Hypaque® Urografin® Renografin® (Winter and Taplin, 1958, and others) sodium 3,5-dipropionylamide 2,4,6-triiodobenzoate, Miokon® (Winter and Taplin, 1958, and others) and 3,5-diiodo-paraaminohippurat (Bianchi, 1961)

Many practical problems which arise when using radiorenography in clinical examinations have been investigated, e.g. position of the patient during the examination (Taplin et al., 1956, Scheer and zum Winkel, 1960, and Abt and Balkus, 1961) the importance of accurate centering of the scintillation detector and exact localization of the kidney (Särborg, 1960) the role of the shielding of the detector (Frohlich et al 1959 Bodfish and Roberts, 1960) and the reproducibility of the test (Spencer et al. 1961)

^{131}I-labelled sodium ortho-iodohippurate

In 1960, according to Nordyke et al. another agent, ^{131}I-labelled sodium ortho-iodohippurate (Radio-Hippuran® and Hipputope®) was available for radiorenographic studies. In preliminary reports these authors and Winter et al., (1960) compared the excretion in urine of ^{131}I labelled ortho-iodohippurate, ^{131}I Diodrast and ^{131}I Miokon, in humans. Labelled ortho-iodohippurate was excreted at a faster rate than the other two compounds. In comparing the radiorenograms, the uptake phase of the radiorenogram curve showed the highest amplitude, when tagged ortho-iodohippurate was used. Nordyke et al., 1960 found that the ^{131}I ortho-iodohippurate renograms from the right and the left kidneys were almost the same in shape and amplitude. This fact would indicate that any influence on one or the other renogram curve, owing to uptake of radioactivity in surrounding organs, especially in the liver did not exist. Even in cases with poorly functioning kidneys the possible compensatory uptake of radioactivity in the liver did not seem to influence the renogram curves. According to these findings, the uptake of radioactivity in the liver should be very low. Determinations of the radioactivity recovered in bile collected from the common bile duct of a patient with normal liver function and with a T tube in ductus choledochus, have shown that the excretion of radioactivity through the liver is also very low. The uptake of radioactivity in the thyroid gland was found to be less than one per cent in two individuals injected with ^{131}I-labelled ortho-iodohippurate (Nordyke and others)

Experimental investigations. In order to verify the suppositions from the preliminary clinical reports indicating low extrarenal excretion of ^{131}I-tagged ortho-iodohippurat some investigations on animals have been performed. Whitley et al. (1961) injected Hipputope into one group of dogs and radioactive Diodrast into another. The results of external measurements as well as of distribution studies

capacity (Tm) was pointed out by Goldring *et al.* (1940) A quantitative measurement of Tm was obtained by administering so large amounts of e.g. Diodrast that the increase in plasma concentration will not be accompanied by a proportional rise of the tubular excretion.

In the above described renal function tests the values were based on chemical analyses of blood and urine samples. It necessitates rather high concentrations of the test substances in order to gain accurate measurements. By introducing isotope technique, chemical analyses can be omitted and very low concentrations of substances can be estimated with a high degree of accuracy The first attempt to use isotope labelled test material in measuring the kidney function was made by Oeser and Billion (1952) They injected the [131]I labelled X ray contrast medium Uroselectan B[n] intravenously and measured its excretion in urine by determining the beta ray activity Thereafter many of the formerly used non radioactive agents for kidney function tests were tagged with different radioisotopes and kidney function studies were performed using isotope technique (Billion and Schlungbaum, 1955 Schlungbaum and Billion, 1955 Block and Burrows, 1958 a, Bergstrom *et al.* 1959 Parker and Beierwaltes, 1960, Boyd and Murdock, 1961 Burbank *et al.*, 1961 Denneberg *et al* 1961 Gott *et al.* 1961 Schwartz and Madeloff 1961 and Winter 1961 a)

Radiorenography

The most important contribution to the study of kidney function with the aid of radioisotope labelled substances was made by Taplin *et al.* in 1956.

Since the gamma radiation has a very low absorption in the air and soft body tissues, the gamma ray intensity emitted from an isotope can be determined at a distance. Taplin *et al* (1956) made use of this fact with [131]I-labelled Diodrast. By means of sensitive scintillation detectors which were connected with ratemeters and graphic recorders, the impulses could be registered against time. With one detector pointed to each kidney and one to the heart area they aimed to register the renal uptake and excretion and the disappearance of [131]I Diodrast from the blood. The time-radioactivity curves, so-called radiorenograms, obtained over the kidneys consisted of three phases a) an initial radioactivity rise, which the authors assumed to represent the first flood of the radioactive agent through the vascular bed of the kidney and surrounding tissues b) a second phase, consisting of a rise of the tracing which may reflect the secretory power of the tubular cells, (the normal radiorenogram reaches a maximum within a short time) thereafter follows c) a third segment, in which a drop in the curve occurs due to excretion into urine and removal of the radioactivity from the exposure of the scintillation detector Taplin *et al* emphasized the clinical value of the new test. Thus, information about some specific qualitative unilateral changes of the kidneys could be obtained. The preliminary works by Taplin *et al* (1956) and Winter (1956) stimulated further investigation with [131]I Diodrast. In experimental studies, some basic theoretical problems of the [131]I Diodrast kidney function test have been considered (Magnusson, 1957 and 1958 Haukenes *et al.* 1958, Winter 1959 de Maria *et al.*, 1960, Denneberg *et al* 1960

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¹³¹I-labelled sodium ortho-iodohippurate

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revealed a greater specificity for renal excretion of Hipputope than of ^{131}I Diodrast. They also found that serial blood sampling and external radiocardiogram curves had the same slopes. Zum Winkel and de Maria (1961) studied the extrarenal excretion of radioactivity after injection of Radio-Hippuran ^{131}I tagged Diodrast, Urografin, and Triopac, in rats. The radioactivity appeared in smaller amounts in the bile when Radio-Hippuran was administered than when the other substances were used. The uptake in the liver and digestive tract was also found to be lower after injection of Radio-Hippuran in comparison with the other test agents. However according to the estimations of radioactivity in blood at 10 and 30 minutes after injection, Radio-Hippuran showed higher values than the other test agents, which fact does not correspond with earlier preliminary clinical experience.

Clinical investigations In recent clinical works, the superiority of ^{131}I labelled sodium ortho-iodohippurate to earlier compounds used in radiorenography has been confirmed by Herron and Barbour (1961) and zum Winkel *et al* (1961)

The sensitivity of ^{131}I-ortho-iodohippurate renography could be increased by a simultaneous injection of para-aminohippuric acid, as shown by zum Winkel *et al.* (1961)

The importance of external measuring over the urinary bladder in order to estimate the urinary output of the radioactivity was pointed out by Herron and Barbour and zum Winkel *et al.* The last mentioned investigators criticized the evaluation of the disappearance of radioactivity from blood by external measurements over the heart area because of the possibility of a part of the radioactivity escaping extravascularly

The early report from Nordyke *et al.* that the liver radioactivity did not influence the right radiorenogram curve does not agree with the findings of Meade and Shy (1961) By performing renography under standard conditions on 78 normal humans they established that the tracing for the right kidney was significantly greater than that for the left, at the maximum of the curves. The possible role of the liver was further evaluated in a patient with a freely draining common bile duct tube. He excreted 0 018 and 0 16 per cent of the given dose in bile at one and 24 hours after the injection of Radio-Hippuran. In several patients the radioactivity over the thyroid gland was measured at 24 hours after the injection and none was detected. In load experiments by addition of hippuric acid they did not find any changes in the amounts of radioactivity excreted into urine by 30 minutes.

The advantage of the radiorenographic technique in detecting unilateral kidney damage with ^{131}I-ortho-iodohippurate, has been pointed out by Nordyke *et al.*, 1960 Winter *et al.* 1960 Corcoran 1961 Herron and Barbour 1961 McDonald, 1961 Hirakawa *et al.* 1961 zum Winkel *et al* 1961 Winter 1961 b and c, Oeff *et al.*, 1961 Maxwell, 1962. In spite of the high renal affinity of the labelled ortho-iodohippurate false positive and false negative results have been reported by Maxwell (1962) who considered that the test served only to detect inequality in kidney function and was not predictive

The aim of the present investigation

Hitherto, external measurement in radiorenography has given mainly qualitative information about the renal accumulation and excretion of the injected radioactive test substances. Since radioisotope labelled compounds with greater specificity for renal excretion are now available, the demand for quantitative analysis of the external measurement has become highly desirable. Proper knowledge of the biokinetic processes of the agent, however must be acquired in order to carry out such evaluations. Relevant data are easily furnished, and in many instances, only obtainable, by animal experiments.

According to earlier investigations and to the author's preliminary experiences ^{131}I-labelled sodium ortho-iodohippurate has a renal specificity greater than other available substances. Therefore this compound has been used in the present study

An important problem is to analyze the purity and stability of the test agent since it is known that substances containing iodine have a tendency to decompose under certain conditions. If this were the case with ^{131}I-tagged ortho-iodohippurate the decomposition products would differ biologically from the original substance and then disturb the evaluation of the kidney function test. The author has given a preliminary report of the results from an examination of the chemical stability of labelled ortho-iodohippurate (Magnusson, 1961) Further investigations on this subject are included in the present work.

A fundamental question to solve in the study of biological behaviour of ^{131}I tagged ortho-iodohippurate is to control how the external measurements reflect the real changes of the radioactivity contents of organs to be checked. In this work, information has been obtained by direct measurement of the radioactivity of organs removed at different time intervals after injection of the labelled compound and comparing this time-concentration curve with the external scintillation curve.

Furthermore, the author has tried to elucidate the relation of the external measurements over certain regions of the body to the hemodynamic kinetics of ^{131}I-tagged ortho-iodohippurate. In this comparison a reference substance has been used to represent the relative size of the vascular bed in the regions of the body over which the external measurement has been performed. A material suitable in this estimation is ^{131}I-labelled serum albumin, which has been injected at an appropriate time after the administration of ^{131}I-labelled ortho-iodohippurate. The real blood radioactivity kinetics has been estimated to give further support to the quantitative evaluations of the external measurements.

Acute cessation of the function of one kidney *e.g.* total occlusion of one renal artery due to embolism may be associated with many diagnostical difficulties. It is usually presupposed that an important decrease of the total renal excretory capacity occurs before the compensatory hyperfunction of the other kidney has developed. According to earlier investigations and to the author's own experiences radiorenography has the capacity to establish the loss of the function of one kidney but the possibility to determine the function of the remaining kidney seems to be of even greater clinical and scientific interest. To analyse this problem the author has performed radiorenographic

examinations on unilaterally nephrectomized animals, before the compensatory hyperfunction of the remaining kidney developed.

It seems to the author that the excretory ways competing with the kidney may play some role in the judging of the excretion of ^{131}I-labelled ortho-iodohippurate in cases of acute ceasing of the function of one kidney. To estimate the extrarenal excretory capacity studies of the distribution and kinetics of ^{131}I labelled ortho-iodohippurate in bilaterally nephrectomized animals have been carried out.

By the subsequent knowledge the author has modified the radiorenographic technique for clinical examinations. Some selected patients with normal, moderately and severely damaged kidney function have been examined with this technique in order to demonstrate its fitness for use in the clinical work.

Radioisotope-labelled substances

All isotope-labelled substances were supplied by Abbott Laboratories, Radio-Pharmaceuticals, Oak Ridge, Tennessee, U.S.A.

^{131}I-labelled sodium ortho-iodohippurate

The role of hippuric acid in some metabolic processes in the mammalian body has long been known. Thus it was early shown that the introduction of benzoic acid or its sodium salt into the animal organism resulted in its conjugation with glycine and in the excretion of hippuric acid or its sodium salt in the urine. In similar fashion it has been found that the administration of ortho-iodobenzoic acid in dogs and rabbits results in the urinary excretion of ortho-iodohippuric acid (Novello *et al.* 1926). This metabolic product, sodium ortho-iodohippurate was examined by Swick, in 1933 as contrast medium in excretion urography. He found a high excretion rate of the administered compound in the urine. In the 1930's the renal excretion mechanism of this substance was studied by means of clearance estimations (Elsom *et al.* 1934—1935 1936, 1937 and Smith *et al.* 1938).

Sodium ortho-iodohippurate, like hippuric acid and many of its derivatives was found to have a high excretion rate in the urine (Smith *et al.* 1945), but the compound eventually went out of use. Nordyke *et al.*, in 1960, however used labelled sodium ortho-iodohippurate in radiorenography according to the method of Taplin *et al.* (1956); thus the compound was "rediscovered" and restored to popularity.

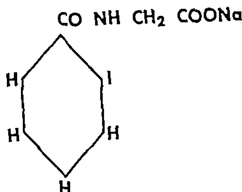


Fig. 1 Sodium ortho-iodohippurate.

Chemically sodium ortho-iodohippurate is $C_9H_7INNaO_3.2H_2O$ and the molecular weight 363.10. Its structural formula is shown in fig. 1. The iodine exists in a stable organically bound state (Swick, 1933) and composes about 35 per cent of the compound (The Dispensatory of the United States of America). The non-radioactive substance is usually prepared by condensation of ortho-iodobenzoic acid with glycine and treatment with sodium hydroxide solution. The crystals are freely soluble in water and this water solution is neutral or faintly alkaline to litmus (Merck Index, 1952).

The first preparation of ^{131}I labelled sodium ortho-iodohippurate was carried out by Tubis *et al.*, in 1960, who developed two methods of exchange reaction

between $^{131}I^-$ in $Na^{131}I$ and the non radioactive iodine atom in sodium ortho-iodohippurate at pH 6.0 and 100° C. The free radioiodohippuric acid was isolated from the sodium salt in the presence of small carrier amounts of iodide. The labelled substance, according to Winter *et al.* (1961) is stable, heat resistant and non toxic. The melting point of the labelled compound was estimated at 173.5 C, which "differed from that reported, in 1926 by Novello Miriam and Sherwin (170 C) but agreed with another source which reported 171—174 C (Tubis *et al.* 1960)." Another method of exchange-labelling of ortho-iodohippuric acid was reported by Scheer and Meier Borst, in 1961 Ortho-iodohippuric acid was labelled with ^{131}I at pH 1.8 and 114 C. After purification by re-crystallization the final product contained less than 1 per cent free ^{131}I

Commercial preparations of ^{131}I labelled sodium ortho-iodohippurate are Radio-Hippuran (Abbott) and Hipputope (Squibb) of which only Radio-Hippuran is available on the Swedish market. It is supplied in sterile and pyrogen free, rubber-stoppered, colourless, transparent glass vials. Each ml of the solution contains 0 2—1.5 mC ^{131}I The specific activity is from 0.2 to 0 7 mC per mg Benzyl alcohol (0.9 per cent) and sodium citrate (2 mg per ml) are added as bacteriostatic and stabilizing agents, respectively Batches of 3—5 mC were supplied.

^{131}I labelled serum albumin

In circulatory studies many radioisotope-labelled substances have been tried, e.g ^{32}P tagged red blood cells in determination of the cardiac output (Nylin and Celander 1950) ^{24}Na in peripheral, regional circulation studies (Kety 1949) and ^{198}Au *in estimation* of the liver blood flow (Vetter *et al* 1954) Labelled proteins were also found to be valuable in circulatory studies. Storaasli *et al.* (1950) determined the blood volume with radioactive iodinated plasma proteins, and Pritchard *et al.* (1952) *utilized* ^{131}I tagged human serum albumin in determination of the cardiac output. This compound is now widely used *in various circulatory* examinations owing to its slow disappearance rate from the circulation (Pritchard *et al* 1955) Some liberation of ^{131}I from the tagged protein, however was early pointed out by Berson *et al.* (1953) The iodolysis, which may invalidate the results, has been attributed to irradiation damage of the proteins (Berson and Yalow 1957 Steinfeld *et al.*, 1958) In order to diminish this irradiation effect the addition of excess albumin *to the radioactive* solution was recommended by Berson and Yalow (1957)

In the present investigation the commercial preparation RISA (Abbott) has been used. It was supplied as a sterile, pyrogen free solution in hermetically-sealed glass vials. This RISA solution contained 140—160 μC ^{131}I and approximately *10 mg albumin per ml.* The "loosely bound radio-iodine was less than 2 per cent. Benzyl alcohol, 0.9 per cent, was added as a bacteriostatic agent and sodium bicarbonate as buffer Batches of 1 mC were supplied.

Radioactive sodium iodide

Carrier free $Na^{131}I$ as a sterile and pyrogen-free solution containing 0 9—2 3 mC *per ml* was used. Benzyl alcohol, 0 9 per cent, and cysteine hydrochloride, 0 2 per cent, were added as preservatives.

Radioisotope and Detection Technique

The isotope

Radio-Hippuran and RISA are labelled with ^{131}I. This isotope emits both beta and gamma rays of several energies. The half-life of ^{131}I is 8.14 days. These properties make it a suitable medium in clinical and experimental investigations.

The identity of the radioactive iodine has been determined by gamma ray spectrometry (fig. 9) and by estimation of the half-life, the results of which were in close accord with the characteristics of of ^{131}I.

Handling of the stock solutions

The stock solutions, received within 3—4 days after the delivery date, were kept in glass bottles, sealed with rubber membranes and stored in the dark in a refrigerator at a temperature of +2° C. Additional human albumin (KABI Ltd, Sweden) was added in amounts of 5 mg to each ml of the stock solution of RISA. All isotope solutions were used within 15—20 days.

Isotope dilutions

In order to administer as nearly as possible the same amount of radioactivity and the same volume in the animal experiments, the stock solutions were diluted with various amounts of physiologic saline. The older the stock solution became, the less dilution was necessary. All isotope dilutions were stored in the dark and at a temperature of +2° C. In the diluted solutions of Radio-Hippuran the content of sodium ortho-iodohippurate varied from 50 to 330 μg per ml. In the diluted solutions of RISA the content of albumin varied from 1.5 to 15 mg per ml.

Standards

The standards from the various solutions of Radio-Hippuran and $Na^{131}I$ were prepared by the following method: by means of an all-glass tuberculin syringe a small amount of the solution was removed and the syringe was immediately weighed on a Mettler semi-micro balance. The contents were ejected into a glass tube (diam., 28 mm, height, 95 mm) containing 12.0 ml water and the syringe was immediately weighed again. The glass tube was sealed with parafilm and used as standard for organs which were checked for radioactivity in the same kind of glass tubes. Usually two to three such standards were prepared from each dilution of the stock solution.

In a similar way standards of the solutions were prepared and kept in plastic tubes containing 2.0 ml water and fitted

for the well-crystal. They were used as standards in radioactivity measurements of bile blood and urine samples. Two to three standards were ordinarily prepared

The glass syringe with its contents of the stock solution was reduced in weight during the preparation of standards. During 30 minutes this weight loss amounted to about 0.1 per cent of the weight of the contents in the syringe. The differences between the standards in the glass tubes were not more than 1.5 per cent of the mean. The variations between the standards in the plastic tubes did not exceed 1 per cent of the mean.

Equipment for external measurement of gamma rays

Two apparatuses, each consisting of a Tracerlab P 20 BQG scintillation detector mounted in a P 25 clinical lead collimator with 24 mm I.D straight boring, and a channel length of 11.2 cm were used. The collimator and detector were held up by a modified RUA-8 Schönander stand. The NaI (Tl) crystal was cylindrical and 1 × 1 in size. The scintillation detector operated in connection with a Tracerlab SC-34 A precision ratemeter at 1,200 volts. The time constant could be set at 0.1 0.5 2.5 and 10 seconds. The impulses from the ratemeter were registered by a Varian G-10 graphic recorder with two paper velocities 51 mm and 6.8 mm per minute.

The shielding effect of the collimator was examined by ^{131}I-scan-curves and isoresponse curves in water with the outer end of the collimator 1 cm from the water surface in order to resemble the conditions under which the external measure-

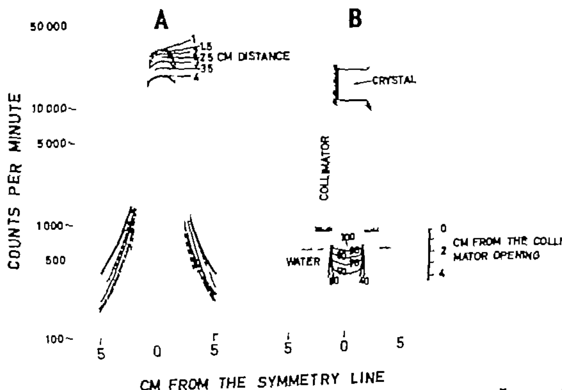


Fig. 2 Scan-curves (A) and isoresponse curves (B) in water obtained from a small source of Radio-Hippuran, recorded with the equipment for the external measurement.

ments are performed in the rats. As shown in fig. 2, the collimator had good lateral resolution but the axial resolution was bad. The isoresponse curves are of the same appearance as found by Kakehi (1959) in studying this type of collimation.

The radiologic center of the crystal used in external measurements was estimated by the inverse-square law and was found to be 17 mm from the end of the detector.

One apparatus for external measurement was used in performing radiorenograms under standard conditions. The reproducibility of the graphic recorder of a Co^{60} standard was examined and found to vary by not more than 2 per cent from the mean value.

Techniques in scintillation measurements of samples

Apparatus

1. A Tracerlab SC-57 low background, well-scintillation detector with a cylindrical thallium-activated sodium iodide crystal 1 × 2" was used. The central well of the crystal was ¾ × 1 in size and able to accomodate a 5 ml sample. The detector was operated together with a Tracerlab SC-33 A 1,000 Scaler at 1,200 volts. The background was 88 ± 3 counts per minute (cpm). About 84 per cent of the total gamma radiation in 2 ml sample was assumed to cross the crystal.

A Tracerlab P 20 BQG scintillation detector with cylindrical thallium-activated sodium iodide crystal, 1" × 1 in 5 cm lead shield was used. The detector operated together with a Tracerlab SC 18-A Superscaler at 1,200 volts. The background was 90 ± 3 cpm. About 16 per cent of the gamma radiation from the sample crossed the crystal. When counting a Co^{60} standard under the same geometrical conditions (147 mm from the end of the detector) as the syringe when performing radiorenograms under standard conditions, the counts per minute did not vary by more than 1 per cent.

The capacity of the registering systems of the two scalers used was tested by measuring different strong radioactivities. A linear relationship was found to exist between the amount of radioactivity and counted impulses up to 1 100,000 cpm.

As a rule the samples were counted for 5 minutes. The radioactivity in samples of blood, bile and urine, which was measured in the well-scintillation detector varied as follows in blood samples between 15,000 and 300 in bile samples between 100,000 and 600, and in urine samples from 300,000 to 100 cpm. The cpm obtained from organs, the radioactivity of which was determined in the P 20 BQG detector was from 900,000 to 50. When the radioactivity was lower than 300 cpm in the well-scintillation detector and 200 in the P-20 BQG detector respectively the counting time was extended to 10 minutes. In some cases when organs with low radioactivity were counted with the P 20 BQG detector the counting time for 1,000 impulses was determined.

Preparation of samples

As a rule, 0.03 ml blood was taken from the tail of the rat in a 0.10 ml pipet. At the end of some animal experiments, when the radioactivity in the blood was low 0.10 ml blood was taken. The contents of the pipet were blown out into 2.0 ml distilled water in a plastic tube. The pipet was carefully rinsed with the solution in

the tube. The radioactivity of blood was measured in the well-scintillation detector

Bile was collected in plastic tubes. The contents of the tubes were diluted with distilled water to 2.0 ml, and the radioactivity measured in the well crystal.

Urine and the rinsing water were collected in plastic tubes. After adjusting the volume to 2.0 ml with distilled water the tubes were counted in the well-scintillation detector

The error in radioactivity measurements in the well-scintillation detector due to divergence from the correct volume, 2.0 ml, was examined (fig 3 A) and was found to be very slight.

Organs and contents of different parts of the digestive tract and the washing solutions were counted in glass tubes as described above. Before the estimation of radioactivity the organs were homogenized by boiling in 10 per cent NaOH for about 30 minutes, after which the volume was adjusted to 12 ml with distilled water The glass tubes were counted in the P 20 BQG detector The glass tubes were placed in the symmetry line of the counting system, usually upon a box 14 mm in height. When the radioactivity of an organ, especially the urinary bladder exceeded 700 000 cpm, control measurements were performed at a distance of 30 mm from the end of the detector The loss in radioactivity upon homogenization of the organs did not exceed 0.5 per cent. In order to determine the influence of differences in volume the same amount of radioactivity was checked in different volumes (fig 3 B) A difference of 1 ml from the 12 ml changed the measured intensity of radioactivity by not more than 3 per cent.

Analysis of the chromatograms and electropherograms

Autoradiography

After careful drying the sheets or strips of the chromatograms and electropherograms were pressed against Gevaert Curix Rapid X ray film. The exposure time was 6—10 days. The films were then developed in Kodak D 163 in dilution 1 1 for 3—4 minutes at room temperature. The development was interrupted in a water bath containing about 2 per cent acetic acid. The fixing of the films was performed for about 30 minutes in Kodak acid fixer After

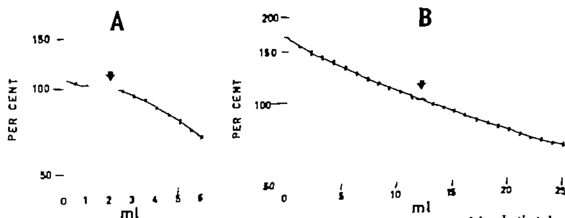


Fig 3. Relation volume-radioactivity of a sample of Radio-Hippuran counted in plastic tubes with a well crystal when 2.0 ml is set at 100 per cent (A) and counted in glass tubes with a 1 × 1 solid crystal when 12 0 ml is set at 100 per cent (B)

fixing, the films were washed in running water for 2—3 hours and then dried in air

Beta ray scanning

Strips of chromatograms and electropherograms were analysed with a chromatogram scanner (Frieseke & Hoepfner 452) in which the strips passed by the windows of two Geiger Tubes (window thickness 1.06 mg/cm² operating voltage, 1,500 volts) with a velocity of 10 mm per minute. The width of the collimator slit could be either 3 or 0.5 mm. The wide slit was usually used only in separation of closely situated fractions was the narrow slit used.

The Geiger tubes were connected with a registering apparatus consisting of a Tracerlab SC-34 A precision ratemeter and a Varian G-10 graphic recorder

Direct measurement of the gamma ray activity in chromatograms and electropherograms

The direct measurement of the chromatograms and the electropherograms was performed by two methods. In the first the strips were cut in equally sized pieces (0.5 or 1 cm broad) at right angles to the direction of the movement. The pieces were counted in the deep well-scintillation detector for 3 minutes. In the second method the whole fractions, the localization of which was determined by autoradiography or β-ray scanning, were cut out and placed in plastic tubes so that the different fractions from the same strip or sheet had the same geometry in the tubes. The radioactivity was then estimated in the well-scintillation counter. The rest of the strips or sheets was also counted for radioactivity in order to determine the total radioactivity

Gamma ray spectrometry

The equipment for gamma ray spectrometry consisted of a Tracerlab RLD-2 gamma scintillation spectrometer detector placed in a Tracerlab P 25 collimator when performing external measurements, and in a lead shield (LKB 3387) in estimations of samples. The detector was connected with a Tracerlab RLI-4G basic pulse height amplifier analyzer. A Tracerlab SC-34 A precision ratemeter was connected with the system for count readings, which were registered by a Varian G-10 graphic recorder

the tube. The radioactivity of blood was measured in the well-scintillation detector

Bile was collected in plastic tubes. The contents of the tubes were diluted with distilled water to 2 0 ml and the radioactivity measured in the well crystal.

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The error in radioactivity measurements in the well-scintillation detector due to divergence from the correct volume, 2 0 ml, was examined (fig 3 A) and was found to be very slight.

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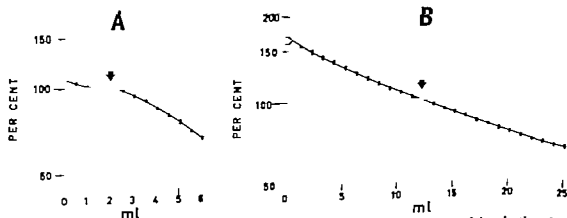


Fig 3 Relation volume-radioactivity of a sample of Radio-Hippuran counted in plastic tubes with a well crystal when 2 0 ml is set at 100 per cent (A) and counted in glass tubes with a 1 × 1 solid crystal when 12.0 ml is set at 100 per cent (B)

Examination methods

Paper chromatography

Paper Whatman filter paper no. 3 MM.

Solvents. I N-butanol—acetic acid—water (4 1 1) pH 2.4

II. N-amyl alcohol—pyridine—water (35 35 : 30) pH 8.7

Apparatus and procedure

One-dimensional chromatography It was found that ascending development separated the various fractions of the test substance adequately The solution was applied as spots or streaks on strips or sheets of paper in amounts of 0.05—0.20 ml along the starting line which was situated about 6 cm from the solvent surface. The papers were attached to a glass frame which was then inserted in a glass tank (22 by 50 cm height 60 cm) containing about 1,000 ml solvent. The chromatographic procedure was performed in the dark at room temperature for 10—15 hours, in some cases for 40 hours.

Two-dimensional k omatography Chromatography in two directions was performed according to the technique of Datta et al. (1950) using solvents I and II The solutions were pplied as spots in amounts of 0.05—0.1 ml. The time development was 6—8 hours in each direction.

The paper strips and sheets were air dried t room temperature

Pape le t phon ns

P per Whatman no. 1

Sol tion I Sodium acetate—acetic acid buffer pH 5.5 ionic strength 0.075

II Primary phosphate—secondary phosphate buffer pH 7.4 ionic strength 0.06.

Apparatus and procedure A. As a rule the electrophoresis was carried out with buffer solution I in a horizontal strip-cabinet containing paper strips (57 by 17 cm) The potential gradient and the time development were 30 V per cm and 30 minutes respectively

B. To exclude any decomposing effect on the examined solutions owing to pH of the solvents in chromatography and of the buffer solution I used in electrophoresis, a control experiment was carried out with electrophoresis with buffer solution II in a horizontal strip-cabinet containing paper strips (50 by 5 cm) The potential gradient and the time development were about 1 1 V per cm and 17 hours respectively

Amounts of 0.010—0.025 ml of the solutions were applied as spots or streaks on the paper After the electrophoretic procedure the papers were air dried at room temperature.

When both electrophoresis and chromatography in two-dimensional separation were performed, the electrophoretic procedure was carried out first. After careful drying the separation was continued with one of the aforementioned chromatographic systems for 6—8 hours.

Staining f th hromatograms and electropherograms

The iodine-containing compound was localized by spraying the paper strips or sheets with 10 per cent solution of phosphotungstic acid in 95 per cent ethanol, after which the areas containing the compound became yellow (Kutt and McDowell, 1962)

CHAPTER IV

Radiochemical analysis of Radio Hippuran and RISA

Radio-Hippuran

The labelled compound was analysed by means of chromatography and electrophoresis with respect to its purity and to its stability under some experimental conditions resembling those under which the preparations may be stored. Separated components were examined by gamma ray spectrometry concerning their isotope identity

Solutions

1 Nine preparations (I—IX) of Radio-Hippuran. Their specific activities were 316—686 μC per mg and concentrations 0.330—2.44 mg per ml. Undiluted stock solutions stored up to 37 days under different conditions were examined.

2 Varying amounts of non-radioactive sodium ortho-iodohippurate (Hopkin & Williams Ltd., England) were added to a stock solution (V) (specific activity 589 μC per mg) This resulted in three solutions, besides the original one, with the following specific activities 300 64 and 6 μC per mg and concentrations 2.68 12 5 and 133 mg per ml, respectively

3 A pure fraction A (see below) was obtained by paper chromatography in solvent I followed by elution with distilled water and concentration by freeze-drying

4 A water solution, containing about 250 μg non-radioactive sodium ortho-iodohippurate per ml was used separately and mixed with a tracer dose of Radio-Hippuran in the chemical identification of fraction A.

5 A water solution of carrier-free $Na^{131}I$ was diluted up to 20 times in order to obtain a solution with the same radioactive concentration as that of the content of fraction B in the examined stock solutions of Radio-Hippuran (see below) The solution of $Na^{131}I$ so obtained was mixed with an equal volume of Radio-Hippuran. The solution of $Na^{131}I$ and the mixture were then used in cross-matching experiments.

Storage of the solutions

a. The solutions in 1 and 2 were stored in the dark at a temperature of about +20 C.

b. Solution 3 and a stock solution (V) with specific activity of 589 μC per mg were stored in glass bottles with a wall thickness of 3 mm and exposed to day light at 20 C. The exposure period included both sunny and cloudy days. Samples of these solutions were kept in the dark at about 20 to serve as controls.

c. Four stock solutions (V VII VIII and IX) were also stored in the dark at a temperature of + 2 C.

Table I Data of hromatographic and electrophoretic examinations of ne recently prepared sol tions of Radio-Hippuran

Separation method	Stock solution no.	Specific activ ty μCi/mg	Concentration mg/ml	Fraction A: Per cent of total radio-activ ty	Fraction A: Rf value in hromato-graphy / Velocity in mm/min in electro-phoresis	Fraction B: Per cent of total radio-activity	Fraction B: Rf-value in chromato-graphy / V locity in mm/min in electro-phoresis	Fraction C: Per cent of total radioactivity	Fraction C: Rf-value in bromato-graphy / V locity in mm/min i electro-phoresis
Chromatography in sol ent I	I	686	1.52	93.99	0.89	6.00	0.21	Included in fraction A — autoradio-graphic finding	1 00
	II	489	1.06	95.61	0.91	4.30	0.19		1.00
	III	408	0.995	94.21	0.90	4.65	0.19		1.00
	IV	610	1 18	90.88	0.92	8.77	0.20	0.08	1.00
	V	589	1.37	92.65	0.91	7.24	0.24	0.56	1.00
	VI	318	2.44	92.19	0.91	6.34	0.24	0.24	1 00
	VII	433	1.57	96.51	0.96	3.36	0.19	0.17	1.00
	VIII	489	0.330	95 67	0.97	3.74	0.14	0.39	1 00
	IX	406	0.513	95.31	0.98	4 15	0.14	Included in fraction A — utoradio-graphic finding	1.00
Chromatography in solvent II	I	686	1.52	92.30	0.50	7.31	0.65	0.30	0.94
	II	489	1.06	93.50	0.47	5.84	0.64	0.33	0.98
	III	408	0.995	93 04	0.51	6.16	0 64	0.37	0.95
	IV	610	1 18	89.96	0.49	9.35	0.61	0.46	0.96
	V	589	1.37	92.05	0 44	7 19	0.53	0.43	0.93
	VI	316	2.44	92.63	0.45	6.87	0.58	0.28	0.96
	VII	433	1.57	93.31	0.47	6.87	0.58	0.42	0.93
	VIII	489	0.330	95.45	0.45	3.31	0.53	0.98	0.92
	IX	406	0.513	93.03	0.40	4 93	0.52	0.77	0.92
Electrophoresis in buffer solution I	IV	610	1 18	91.20	2.5	8.19	6.7	0.59	0.9
	V	589	1 37	93.20	3 0	6.36	7.8	0 44	1.2
	VI	316	2.44	93.05	2.0	6.66	6.5	0.52	0.7
	VII	433	1.57	95.93	2 1	3.37	3.9	0.66	0.7
	VIII	489	0.330	95.66	2.0	3.17	5.8	1 10	0.8
	IX	406	0.513	94 74	2.0	3.95	5.1	0.92	1.0

Radioactivity measurements of the paper chromatograms and electropherograms

The localization of the radioactivity was estimated by autoradiography and beta scanning. The radioactive spots were quantitatively determined by counting in the deep well scintillation detector as described in Chapter III.

Gamma ray spectrometry

The stock solution of Radio-Hippuran as well as the two most radioactive spots on the paper electropherogram were analysed by gamma ray spectrometry. A Co^{60} source was used as reference. The window width was 5 volts.

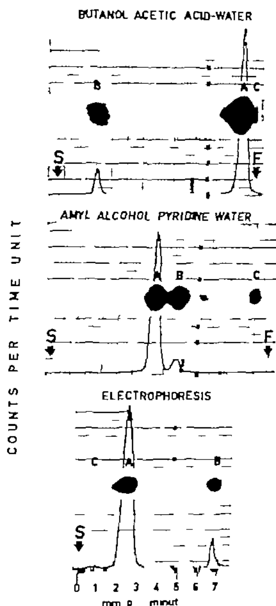


Fig 4 Paper chromatography with two different systems and electrophoresis in buffer solution I of a recently prepared stock solution of Radio-Hippuran. Autoradiograms and scan-curves of the paper strips are shown. S = starting point. F = solvent front. A, B, and C refer to the fractions presented in the text.

Results

Recently prepared Radio-Hippuran

In one-dimensional separation by the two chromatographic systems and by electrophoresis with the buffer I a number of fractions appeared containing varying amounts of radioactivity. The result of analysing one of the stock solutions with autoradiography and beta scanning of the chromatograms and electropherogram is presented in fig 4.

The three main radioactive components are presented in table I which gives their content of radioactivity, their Rf values in the two chromatographic conditions, and velocities in the electrophoretic system I. The most radioactive component in each system was denoted by A, the component with the radioactivity next in size by B and so on. In chromatography with solvent II some small fractions, other than the three presented above, appeared rather regularly with the following Rf values: 0.12—0.14, 0.36—0.38 and 1.00. Their radioactivity did not exceed 0.2 per cent in any of them, and the sum of these fractions, together with some other irregularly occurring components, did not exceed 0.8 per cent of the total radioactivity. As shown in table I a rather good agree-

Table II Mean values and standard deviations of the content of fractions A and B obtained by examination of stock solution of Radio-Hippuran ten times within two days in two chromatographic and two eletrophoretic systems

Separation technique	Fraction A in per cent of the total radioactivity	Fraction B in per cent of the total radioactivity
Chromatography with solvent I	91 19±0.56	8.48±0.52
Chromatography with solvent II	90 42±0.35	7.68±0.27
Electrophoresis with buffer solution I	91 42±0.28	7.95±0.29
Electrophoresis with buffer solution II	91 44±0.32	7 77±0.38

A decrease of component A and a corresponding increase of component B were observed by all separation methods and in all stock solutions examined. No new fractions were detected. The disappearance rate of fraction A varied in the different preparations.

The highest rate of transformation of fraction A to fraction B was mainly found in solutions with the highest specific activity The stock solution with the specific activity of 489 μC per mg (VIII) was an exception. In this solution the diminishing of fraction A occurred slower than expected. The concentration of sodium ortho-iodohippurate was, however considerably lower than that of the other stock solutions examined.

The effect of adding non-radioactive sodium ortho-iodohippurate to the radioactive substance

By adding various amounts of non-radioactive sodium ortho-iodohippurate to the stock solution the disintegration process was diminished as shown in fig. 6 B. The lower the specific activity the greater retardation of the disintegration process. The addition of large amounts of carrier even caused a significant increase of component A with a corresponding decrease of component B, probably indicating a synthesis of fraction A. It was established by autoradiography that no new fractions appeared when the carrier was added. From a stock solution with high specific activity a solution with a specific activity of 900 μC per mg was made by adding non-radioactive substance This solution was decomposed at about the same rate as a stock solution with an original specific activity of 316 μC per mg

The influence of temperature on the decomposition process

Fig 6 C shows the composition of one stock solution (V) stored in the dark at a temperature of + 20° C and of + 2 C. A significantly higher rate of transformation of component A to component B was observed at + 20 C than at + 2 C. Of the three other preparations of Radio-Hippuran (VII, VIII and IX) two (VII and IX) showed a tendency to slower disintegration rate at +2 C than at room temperature while the third one (VIII) did not. These three solutions, especially no. VIII had, however a very slow decomposition rate even at room temperature

ment existed between the radioactive content of the components with the same designation separated in the different systems.

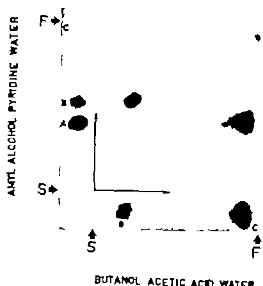


Fig 5 A Autoradiogram of two-dimensional paper chromatography of Radio-Hippuran. Autoradiograms of one-dimensional paper chromatographies with the corresponding solvents are shown under and to the left. Starting point at S and at the crossing of the arrows. Solvent front at F



Fig 5 B Autoradiogram of one-dimensional paper chromatography combined with electrophoresis of Radio-Hippuran. Autoradiograms of electrophoresis and of one-dimensional chromatography are seen under and to the left, respectively. Starting point at S and at the crossing of the arrows. Solvent front at F

Electrophoresis with buffer solution II separated Radio-Hippuran in a similar way as with buffer solution I. Thus, three components appeared, the relative velocities and radioactivity content of which agreed fairly well with those found in electrophoresis with solution I.

By two-dimensional separation technique it was also verified that components A, B and C coincided (fig 5). In this way it was also established that the small fractions appearing in chromatography with solvent II derived mainly from fraction A.

The reproducibility of the separation methods

In order to examine the statistical significance of the reproducibility of the separation methods, ten estimations of the fractions A and B in a stock solution of Radio-Hippuran were performed within two days in each of the two chromatographic systems and in electrophoresis with buffer solution I. To control the effect of pH in the separation of solutions, the same number of estimations was carried out by electrophoresis with buffer solution II

The results are presented in table II. Good agreement existed between the content of the two biggest fractions separated in the four systems. Significantly lower value ($P < 0.01$) of fraction A in chromatography with solvent II was, however observed

Changes during storage of the solutions at room temperature

The changes of the composition of six preparations of Radio-Hippuran, stored in the dark at + 20 C, were followed by examining samples at different time intervals. The results are shown in fig 6 A.



Fig 7 Change of content of fraction A with time when chromatographically purified fraction A is stored in the dark and when stored in daylight. Sunny days are marked with ○

The [illegible] *ct of light e posure* [illegible] *actio A and Radio-H ppuran*

Two samples of pure fraction A, one stored in the dark and the other exposed to daylight, were subjected to chromatography at different time intervals (fig 7) The results agreed well with those obtained by handling a stock solution of Radio-Hippuran in a similar way and testing it with chromatography and electrophoresis. A higher rate of transformation of fraction A to B was observed in daylight than in the dark at room temperature. During sunny days this rate was still higher but that this effect may be due to an increase in temperature cannot be excluded. The decrease of component A coincided with an increase of fraction B, while other components remained unchanged and no new ones appeared according to autoradiographic examinations (fig 8)

The hemical identity [illegible] *fractions A and B*

Fraction A Radio-Hippuran and non-radioactive sodium ortho-iodohippurate were studied with chromatography and electrophoresis in order to examine whether they behaved in a similar way or not. Solutions mentioned in 4 were used. Earlier described separation methods, one-dimensional hromatography and electro-

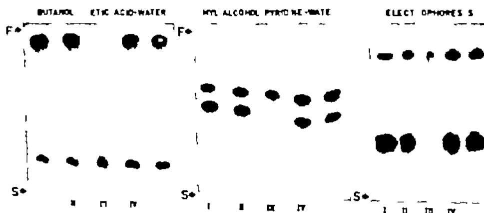


Fig 8. Autoradiograms of one-dimensional paper chromatography in two sol ents and of electrophoresis of stock solution of Radio-Hippuran, stored in the dark for 5 days (I), stored in daylight for 5 days (II) Radioacti e iodide sodium iodide (III). Autoradiograms (IV) and (V) refer to the cross-matching of (I) and (III) and of (II) and (III), respectively S = starting point. F = solvent front.

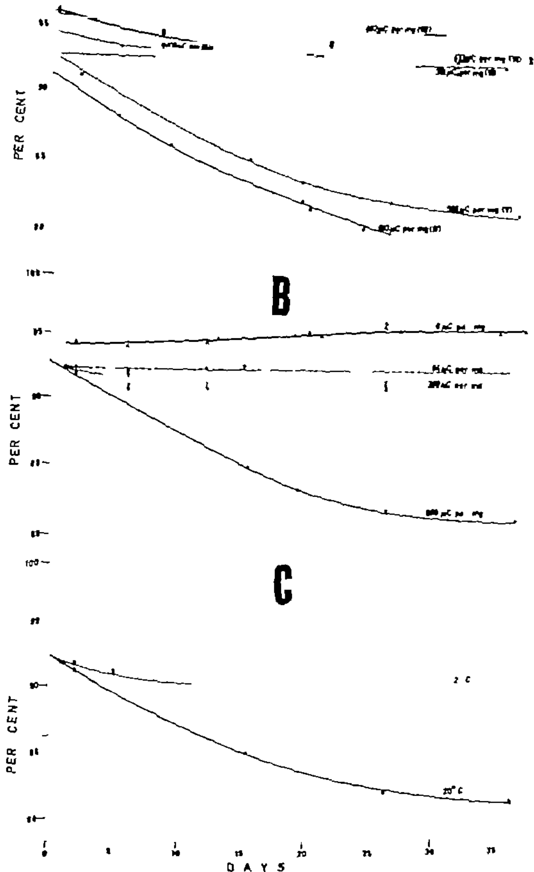


Fig. 6. Change of content of fraction A with time in six different stock solutions (A) in four solutions with various specific radioactivity three of which were prepared from a stock solution by adding stable sodium orthoiodohippurate (B) in a stock solution stored at + 20 C and at + 2 C (C). The solutions in A and B were stored at room temperature. The numbers of μC per mg refer to the specific radioactivity at the delivery date, 9—4 days before zero time. Roman numerals in brackets denote the different stock solutions, see table I

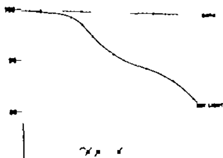


Fig 7 Change of content of fraction A with time when chromatographically purified fraction A is stored in the dark and when stored in daylight. Sunny days are marked with

The effect of light exposure of fraction A and Radio-Hippuran

Two samples of pure fraction A, one stored in the dark and the other exposed to daylight, were subjected to chromatography at different time intervals (fig 7) The results agreed well with those obtained by handling a stock solution of Radio-Hippuran in a similar way and testing it with chromatography and electrophoresis. A higher rate of transformation of fraction A to B was observed in daylight than in the dark at room temperature. During sunny days this rate was still higher but that this effect may be due to an increase in temperature cannot be excluded. The decrease of component A coincided with an increase of fraction B, while other components remained unchanged and no new ones appeared according to autoradiographic examinations (fig 8)

The chemical identity of fractions A and B

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Fig 8 Autoradiograms of one-dimensional paper chromatography in two solvents and of electrophoresis of stock solution of Radio-Hippuran, stored in the dark for 5 days (I) stored in daylight for 5 days (II) Radioactive iodide as sodium iodide (III) Autoradiograms (IV) and (V) refer to the cross-matching of (I) and (III) and of (II) and (III) respectively S = starting point. F = solvent front.

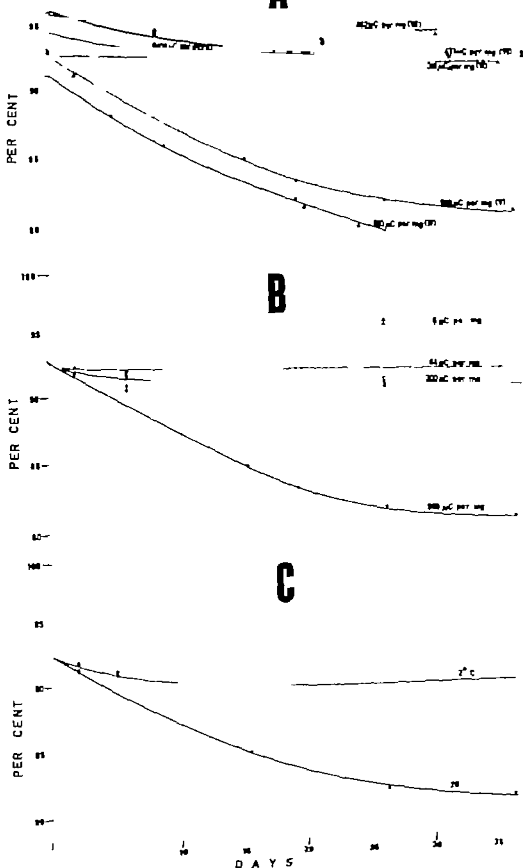


Fig 6. Change of content of fraction A with *time* in six different stock solutions (A) In four solutions with various specific radioactivity three of which were prepared from a stock sol tion by adding stable sodium ortholodohippurate (B) in a stock solution stored t + 20 C and at + 2 C (C) The solutions in *A* and *B* were tored at room temperature. The numbers of µC per mg refer to the specific radioactivity at the delivery date 3—4 days before zero time. Roman numerals in brackets denote the different stock solutions, see table I

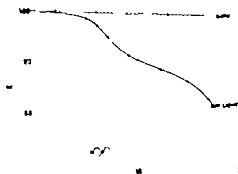


Fig 7 Change of content of fraction A with time when chromatographically purified fraction A is stored in the dark and when stored in daylight. Sunny days are marked with ○⁄

The effect of light exposure of fraction A and Radio-Hippuran

Two samples of pure fraction A, one stored in the dark and the other exposed to daylight, were subjected to chromatography at different time intervals (fig 7) The results agreed well with those obtained by handling stock solution of Radio-Hippuran in a similar way and testing it with chromatography and electrophoresis. A higher rate of transformation of fraction A to B was observed in daylight than in the dark at room temperature. During sunny days this rate was still higher but that this effect may be due to an increase in temperature cannot be excluded. The decrease of component A coincided with an increase of fraction B while other components remained unchanged and no new ones appeared according to autoradiographic examinations (fig. 8)

The chemical identity of fractions A and B

Fraction A Radio-Hippuran and non-radioactive sodium ortho-iodohippurate were studied with chromatography and electrophoresis in order to examine whether they behaved in a similar way or not. Solutions mentioned in 4 were used. Earlier described separation methods, one-dimensional chromatography and electro-

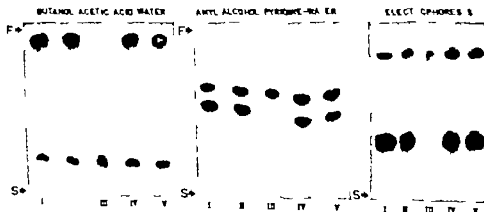


Fig 8. Autoradiograms of one-dimensional paper chromatography in two solvents and of electrophoresis of stock solution of Radio-Hippuran, stored in the dark for 5 days (I) stored in daylight for 5 days (II) Radioactive iodide as sodium iodide (III). Autoradiograms (IV) and (V) refer to the cross-matching of (I) and (III) and of (II) and (III) respectively S = starting point. F = solvent front.

phoresis as well as two-dimensional separation by combining chromatography and electrophoresis, were applied. Autoradiograms were made from radioactive paper strips and sheets. The spots containing iodine compounds were visualized by spraying with phosphotungstic acid.

The blackening of the autoradiograms referring to fraction A coincided in all methods with the yellow spot containing the non radioactive ortho-iodohippurate.

Fraction B Since Radio-Hippuran is prepared by an exchange reaction between non radioactive ortho-iodohippurate and $^{131}I^-$ it is suspected that some radioactive iodide may remain in Radio-Hippuran as an impurity Therefore, the behaviour of $^{131}I^-$ was studied in the different chromatographic and electrophoretic systems.

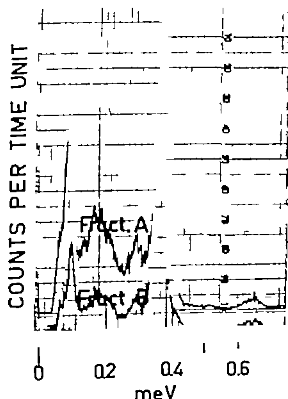


Fig 9 Gamma ray spectrometry of fractions A and B isolated from Radio-Hippuran.

When $^{131}I^-$ was examined separately in the two chromatographic systems and in electrophoresis with buffer solution I the radioactivity appeared as a single spot. In chromatography the Rf values were about 0 20 and 0.58 when using solvents I and II respectively The velocity of $^{131}I^-$ in electrophoresis amounted to about 6.8 mm per minute. Thus, the behaviour of $^{131}I^-$ was similar to component B in the three separation methods. In cross-matching experiments with $Na^{131}I$ added to Radio-Hippuran an increase in the radioactivity of component B occurred corresponding to the amount of added ^{131}I. In the autoradiograms of one-dimensional chromatograms and electropherograms (fig 8) as well as of the two-dimensional chromatograms no new components appeared.

Gamma ray spectrometry

The gamma ray spectrograms of the stock solution of Radio-Hippuran and of the separated components A and B had identical appearances and showed the pattern of ^{131}I (fig 9)

RISA

Previous investigations with ^{131}I-labelled proteins (Berson *et al* 1953) have revealed liberation of free ^{131}I from the tagged material which fact may reduce the usefulness of the results obtained in experimental and clinical investigations with these compounds. In order to estimate the amount of free ^{131}I in a RISA solution the following examinations were performed.

From a fresh solution of RISA a sample was removed, diluted five times with physiologic saline, and immediately ex

amined as described below Human serum albumin was added to the stock solution, which was then stored at a temperature of +2 in the dark. After 19 days a sample was drawn from the RISA solution and diluted twice with physiologic saline and examined as follows

1 *Dialysis.* 2.5 ml of the diluted samples placed in Cellophane bags (36 by 32 Dextar) were dialyzed for 48 hours against 100 ml isotonic phosphate buffer at pH 7.4 and at +4 C. After the dialysis the bags and the buffer solutions were checked for radioactivity

2. *Ultrafiltration.* 1 ml of the diluted samples transferred to collodion tubings (Membrangesellschaft, Göttingen, Germany) was filtered under a pressure of about 40 mm Hg and at +4 C against an isotonic phosphate buffer of pH 7.4 After 15 hours the radioactivity of the tubings and the buffer was measured.

3 *Electrophoresis.* Electrophoresis was carried out as described in the examination of Radio-Hippuran with buffer solution II. 0.010—0.025 ml of the diluted samples were applied to the paper strips. The radioactivity was localized by autoradiography and beta scanning and determined by direct measurements of pieces of the strips. Some strips were stained with amido black

Results

1 *Dialysis.* The radioactivity of the samples from the recently prepared RISA and from that stored for 19 days was reduced by 1.7 and 1.5 per cent respectively

2 *Ultrafiltration.* The loss of radioactivity from samples identical to those in 1 amounted to 1.3 and 0.9 per cent, respectively

3 *Electrophoresis.* Besides a component containing most of the added radioactivity and corresponding to the albumin fraction, another component appeared, the velocity of which was similar to that of $^{131}I^-$ This component contained 1.3 and 0.7 per cent of the radioactivity in the samples from the fresh and from the 19 day old solutions of RISA, respectively

Discussion

Radio-Hippuran

What previously has been mentioned as regards the chemical stability of organic iodine compounds, seems to be applicable also to Radio-Hippuran. Thus, by performing ascending chromatography and electrophoresis it could be demonstrated that different preparations of Radio-Hippuran contain regularly three components with varying radioactive content (Magnusson, 1961) The good agreement between their radioactive content in the different separation methods, verifies that the various components can not be explained by a disintegration process caused by the solvents used in the chromatography (except for some small components appearing when using solvent II) This must instead depend on the presence of different ^{131}I-active compounds of Radio-Hippuran. The statistical investigation of the reproducibility of the four separation methods shown in table II, in which a very good agreement exists between fractions A and B, give more support to this hypothesis. From the values in table II the conclusion can be drawn that changes of pH between 4 and 8.7 do not cause any important decomposition of Radio-Hippuran.

A slight but significantly ($P < 0.01$)

lower value of the content of fraction A was observed when chromatography with solvent II was used (table II) This is also the impression obtained when comparing the results of the examination of the different stock solutions (table I) The two-dimensional chromatography confirms that this finding may depend on a slight decomposition of component A in solvent II

The identical behaviour of fraction A in Radio-Hippuran and of non radioactive sodium ortho-iodohippurate in the chromatographic and electrophoretic systems, supports the hypothesis that they agree as to the chemical structure. Further investigations on the chemical nature of fraction A are in progress (Magnusson, 1962 a)

The cross-matching experiments verified that the main impurity (fraction B) and $^{131}I^{-}$ behaved similarly in three different systems. Thus, it is very probable that fraction B is identical to $^{131}I^{-}$

The impurity and instability of ^{131}I labelled sodium ortho-iodohippurate have been reported by other investigators. Thus, Burbank *et al.* (1961) performed ascending chromatography of ^{131}I labelled sodium orto-iodohippurate on Whatman no. 1 paper in n-butanol saturated with 1 N acetic acid. They found that the tagged compound divided into two fractions, one containing 100—92.6 and the other 0 0—7 4 per cent, with Rf values of 0 84 and 0.20 respectively Free radioactive iodide moved with an Rf value of 0 19 Scheer and Meier Borst (1961) reported that free ^{131}I in solutions of radioactive sodium ortho-iodohippurate increased with time.

The continuous transformation of component A to B during storage cannot be explained *only* by a single cause according to the results of the present investigation. The *different* rate of decomposition of component A with specific activities speaks in favour of the hypothesis that the decomposition may to a part, be explained by autoradiolysis. Exposure to light and elevation of temperature were shown to increase the rate of decomposition. *The finding that fresh stock solutions* of Radio-Hippuran with rather low specific activities contained $^{131}I^{-}$ in such large amounts as up to 9 4 per cent can also be explained by assuming that a large quantity of $^{131}I^{-}$ can remain from the preparation process, which is an exchange reaction between non-radioactive sodium ortho-iodohippurate and $^{131}I^{-}$ at pH 6.0 and 100 C The results of the present investigation are not in agreement with the investigations of Swick (1933) who found that "iodine exists in a stable organically bound state" in non radioactive sodium ortho-iodohippurate and Winter *et al* (1961) who found that ^{131}I-labelled *sodium ortho-iodohippurate was stable* and heat resistant" The distribution and kinetics of the separated fractions A and B were studied in rats (Chap. VII) The clinical consequences of the appearance of the impurities in Radio-Hippuran are discussed in Chapter X.

RISA

As was suspected from earlier investigations, free ^{131}I was also detected in this preparation of ^{131}I labelled albumin. By adding albumin to the RISA solution as recommended by Berson and Yalow (1957) the amounts of free ^{131}I were however kept so small (less than 2 per cent) that, in the *in vivo* experiments, the errors due to the presence of this component were considered to be negligible.

Summary

Radio-Hippuran

1 Chromatographic and electrophoretic analyses of recently prepared Radio-Hippuran (Abbott) have indicated the occurrence of three fractions, of which the biggest (A) contained 90.0—96.5 per cent, the fraction next in size (B) amounted to 3.2—9.4 per cent, and a small fraction (C) consisted of 0.1—1.2 per cent of the total radioactivity

2. Gamma ray spectrometry of the radiation from components A and B showed the pattern of ^{131}I

3. Fraction A and non-radioactive sodium ortho-iodohippurate behaved similarly in the chromatographic and electrophoretic systems used.

4 Fraction B behaved similarly to $^{131}I^-$

5. During storage of Radio-Hippuran the relative amount of fraction A decreased and at the same time an almost corresponding rise of the relative content of fraction B was seen. Thus, a continuous liberation of $^{131}I^-$ from ^{131}I-labelled sodium ortho-iodohippurate was indicated.

6. The decomposition process occurred at a slower rate in solutions with low specific activity than in those with high specific activity and in solutions with low rather than high concentration of ortho-iodohippurate. These results indicate that the disintegration process can partly be explained by autoradiolysis.

7 Exposure to light and heat increase the rate of the decomposition process of Radio-Hippuran.

RISA

8. Examinations by dialysis, ultrafiltration, and electrophoresis of a recently prepared RISA solution and a 19 day old RISA solution to which albumin had been added, revealed that the amount of free ^{131}I did not exceed 2 per cent of the total radioactivity

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CHAPTER V

Affinity of Radio-Hippuran to various blood constituents *in vitro*

Radio-Hippuran used in clinical studies is administered in the blood, which thus forms the primary compartment for the biological kinetics. Substances can be transported by the hemodynamic system in different ways, e.g. bound to proteins, incorporated in the red blood cells, or unbound in plasma. It is therefore of fundamental interest to elucidate on the affinity of Radio-Hippuran to various blood constituents.

Serum

Freshly drawn rat serum and Radio-Hippuran were mixed to obtain concentrations of 0.04, 0.4, 1 and 10 mg ortho-iodohippurate per 100 ml serum. The mixtures were allowed to stand at room temperature for about 30 minutes, and were then examined with respect to the protein binding capacity according to the following methods:

1. *Protein precipitation.* To 0.5 ml serum-Radio-Hippuran mixture in a plastic tube 5 ml of 5 per cent trichloroacetic acid (TCA) were added. The mixture was measured in the deep well scintillation counter. Ten minutes after the addition of the TCA solution the mixture was filtered through Whatman no. 3 paper. The plastic tube and the precipitate were washed four times with 5 ml portions of TCA. The filter paper with the precipitate was then transferred to the plastic tube and the volume adjusted to 5.5 ml. The plastic tube with its contents was again counted in the scintillation counter.

2. *Dialysis.* 2 ml of serum Radio-Hippuran mixture were placed in a Cellophane bag (36 by 32 Dextar). After radioactivity measurement in the deep well-scintillation detector, the bag was dialyzed against 100 ml isotonic phosphate buffer at pH 7.4 and +4° C. After 24, 48, 72, and 96 hours the bag and 2 ml of the buffer were counted separately in the scintillation counter. At 48 and 72 hours the phosphate buffer solutions were replaced with fresh buffer solutions.

3. *Ultrafiltration.* 1 ml serum-Radio-Hippuran mixture was transferred to a collodion tubing (Membranfiltergesellschaft, Göttingen, Germany) which was checked in the well counter and thereafter subjected to continuous suction of 40 mm Hg in an isotonic phosphate buffer solution of pH 7.4 and +4° C for 15 hours. At this time the buffer solution was replaced with fresh buffer and

the filtration continued for another 24 hours. The contents of the tubing were then diluted with 1 ml isotonic neutral phosphate buffer and the suction was continued for another 2 hours. The radioactivity of the tubing was measured at 15 39 and 41 hours after the beginning of the suction.

4 Elect phoresis. The electrophoresis was carried out with Whatman no. 1 paper strips (50 by 7 cm) in a horizontal strip-cabinet. Three electrophoretic solutions with different pH were used

1) Barbital buffer pH 8.6, ionic strength 0.06. Operating time 20 hours, potential gradient of about 2 volt per cm 2) Sorensen s phosphate buffer pH 6.0, ionic strength 0 06, operating time 16 hours, potential gradient of about 2 volt per cm and 3) Sorensen s phosphate buffer pH 7 4 ionic strength 0.06, operating time 16 hours and potential gradient of about 2 volt per cm. About 0.025 ml of a sol tion of Radio-Hippuran and of a mixture of serum and the radioactive agent were p-plied to the strips. The radioactivity was localized by autoradiography beta scanning or direct measurement of pieces of the strips. Some strips were stained with amido black.

Results

1 Protein precipitation. The radioactiv ty of the precipitate amounted to 0.4—0 8 per cent of the total radioactivity in the mixture of serum and Radio-Hippuran. No increased tendency of the protein to bind Radio-Hippuran at low serum concentration was detected.

2 Dialysis. The ratios of ^{131}I in serum to that in buffer averaged 2.8 after 24 hours the ratios were 3.0 and 2.4 respec tively t concentrations of 0 04 and 10 mg

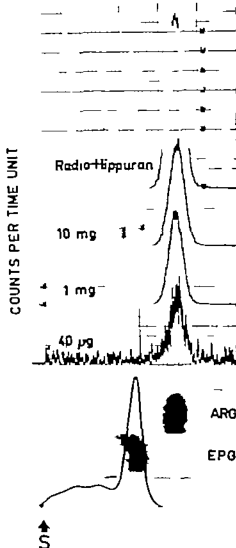


Fig 10. Electropherogram (EPG) and corresponding utoradiogram (ARG) of a mixture of serum and Radio-Hippuran. The four upper scan-curves refer to the radioactivity of the stock solution of Radio-Hippuran alone and of different concentrations of this substance mixed with serum (10, 1 and 0.04 mg per 100 ml serum). The fifth scan-curve gives the protein concentration of the electropherogram. S = starting point. Ordinate axis = protein or radioactivity concentration. The pH of the buffer solution = 7 4.

3— I II

ortho-iodohippurate per 100 ml. After 48 hours the ratios averaged 1.9 with a ratio of 2 0 at the lowest and highest serum concentrations of ortho-iodohippurate. At 72 and 96 hours, 0.9—1.5 and 0.5—0 7 per cent respectively of the total radioactivity remained in the bags.

3 Ultrafiltration The radioactivity in the collodion tubings diminished continuously during the suction. At 15 39 and 41 hours, respectively 5—13 1.3—3 0 and 0 4—0 7 per cent of the total radioactivity remained in the tubings.

4 Electrophoresis In the three electrophoretic buffer solutions no radioactivity was detected in the protein fractions. The Radio-Hippuran added ran faster than the albumin to the anode and moved at the same velocity as Radio-Hippuran in water (fig 10)

Red blood cells

The affinity of Radio-Hippuran to the red blood cells *in vitro* was studied with the following technique

Radio-Hippuran, containing 90.9 per cent of fraction A, was added in a tracer and a carrier dose of 0.3 and 10 mg ortho-iodohippurate per 100 ml whole blood to each of two freshly drawn whole blood samples of 2 ml (hematocrit, 40 per cent) The mixtures were gently shaken and stored at + 37 C. Samples of the mixtures were obtained by filling polyethylene catheters with about 0.1 ml. The polyethylene catheter was then centrifuged for 10 minutes at 2,500 *g* The parts of the catheters containing the red blood cells and the plasma were separated and the radioactivity of each part checked in the deep well-scintillation counter The time interval between the administration of Radio-Hippuran and the beginning of the centrifugation of the catheter was registered. The velocity of immigration of iodide ions into the red blood cells was studied in a similar way by adding a tracer dose of $^{131}I^-$ to freshly drawn whole blood.

The firmness of the erythrocyte binding of Radio-Hippuran was examined by repeated washing of the red blood cells with physiologic saline. 2 ml of freshly drawn whole blood were incubated with the radioactive substance in a dose of 0.5 mg ortho-iodohippurate per 100 ml whole blood at + 37 C for two hours, after which time the mixture was centrifuged for 10 minutes at 1 000 *g* The plasma was removed and the red blood cells were counted in the deep well-scintillation counter 4 ml physiologic saline were then

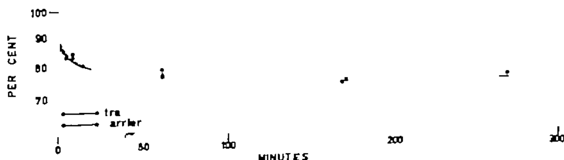


Fig 11 Change of content of Radio-Hippuran in serum with time when tracer and carrier amounts of Radio-Hippuran are mixed with blood *in vitro*.

added, the mixture shaken gently and again centrifuged. After removal of the washing solution the red blood cells were again counted. This procedure was repeated five times.

Results

The results of the examination of the migration velocity of ^{131}I into the red blood cells is shown in fig. 11 where the content of ^{131}I in the plasma is expressed in per cent of the total radioactivity of the polyethylene catheter. The results of the tracer and carrier experiments were on the whole similar.

A rapid decrease of the plasma radioactivity occurred during the first 5 minutes, and an approximate equilibrium was reached at a rate of about 8 per cent per minute. At 5 minutes about 15 per cent of the radioactivity was found in the

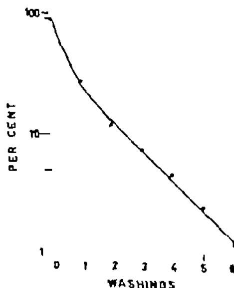


Fig. 12. Per cent of radioactivity remaining in red blood cells after repeated washings of the cells with physiologic saline after mixing whole blood and Radio-Hippuran *in vitro*.

erythrocyte column. Then followed a slow diminishing of the ^{131}I from the plasma. In the experiment with mixing ^{131}I^{-} and whole blood a prompt equilibrium occurred between the content of ^{131}I in the red blood cells and in the plasma with about 52 per cent remaining in the plasma.

The result of washing the red blood cells is shown in fig. 12. The radioactivity of the erythrocytes after each washing is rendered in per cent of the radioactivity in the red blood cells after the first centrifugation. After the first washing procedure a logarithmic decrease of the ^{131}I in the erythrocytes occurred at a rate of about 50 per cent per washing.

Hemoglobin

Two samples of 2 ml each of a solution, containing 2 g of rat oxyhemoglobin per 100 ml distilled water were incubated for two hours at room temperature with Radio-Hippuran in a tracer and a carrier dose of 0.1 and 10 mg ortho-iodohippurate per 100 ml respectively. The binding of Radio-Hippuran to oxyhemoglobin was then examined in two ways.

1. Protein precipitation. 0.5 ml of each solution were treated with a 5 per cent trichloroacetic acid as described above in the examination of the affinity of Radio-Hippuran to serum proteins.

2. *Electrophoresis.* 0.025 ml of each mixture were analysed by electrophoresis with a phosphate buffer of pH 7.4 as described above. The radioactivity was localized by autoradiography, beta scanning and direct measurement of pieces of the strips.

Results

By protein precipitation it was estimated that 2.7 and 1.6 per cent, respectively of the added radioactivity were found in

the precipitate in the tracer and carrier experiments. No blackening of the autoradiograms and no beta ray activity was observed on the place of oxyhemoglobin in the electrophoretic strips. Neither by direct measurement of the strips could any ^{131}I be detected in the oxyhemoglobin fraction.

Discussion

The results obtained by the various methods to estimate the protein binding of Radio-Hippuran corresponded well to each other. They have revealed that no or only small amounts of Radio-Hippuran combine with the serum proteins. They are in good accord with the findings of the conditions *in vivo* (see Chap. VII). The results of earlier investigations concerning the protein binding of non radioactive ortho-iodohippurate disagree. Thus, Elsom *et al.* who in 1936 studied the excretory mechanism of Skiodan, Hippuran, and Diodrast, assumed that these iodine compounds existed in plasma wholly in a filtrable form and based this assumption on the fact that "ultrafiltration through cellophane membrane (no. 450) of horse serum, to which the different compounds were added in widely varying amounts, yielded filtrates which had the same iodine content as the original solution. To the contrary Smith and Smith (1938) reported that, according to experiences from *in vitro* experiments, the three above mentioned compounds were not completely ultrafiltrable from human or dog plasma or from horse serum. The amounts of these substances bound to protein were relatively larger at low than at high serum and plasma concentrations, and followed approximately exponential build-up curves. The ability of rat serum to bind ortho-iodohippurate has not previously been investigated. In 1960 Block and Burrows estimated the protein binding of ^{131}I Diodrast in man by equilibrium dialysis experiments. At serum concentration of ^{131}I labelled Diodrast from 0.01 to 500 mg per 100 ml, the serum to buffer ratio of ^{131}I was nearly constant. An increase of the ratio occurred at serum concentrations below 0.01 mg per 100 ml, indicating a relative increase of the content of non-dialysable ^{131}I labelled Diodrast.

When the corresponding serum concentrations of ortho-iodohippurate and Diodrast were compared, the present results and those reported by Block and Burrows agreed rather well. The conclusion that no considerable amounts of non-dialysable Radio-Hippuran exists in the examined serum concentrations, can justifiably be drawn. The observation of the appearance of Radio-Hippuran in a free form in the electrophoretic systems is in accordance with the results of Bennhold *et al.* (1950). By means of free electrophoresis in barbital buffer they noticed that test agents excreted through the kidneys (Perabrodil, Uroselectan B, Neo-iopax, indigo carmine, phenol red, and Victoria violet 4 BS) moved as free substances indicating that, if a binding to the blood proteins exists, this must be very loose.

The biological consequences of the fact that Radio-Hippuran exists in an unbound state in the blood plasma are elucidated in Chapter VII.

In the present investigation it was established that Radio-Hippuran in considerable amounts penetrated the rat erythrocytes *in vitro*. Partly the high radioactivity content in the red blood cells could be ex

plained by penetrated $^{131}I^-$ which, from results from migration experiments of $^{131}I^-$ into red blood cells, could be estimated at 3.6 per cent of the total radioactivity. Thus, most of the radioactivity in the erythrocytes must derive from the fraction A of the commercial preparation of Radio-Hippuran. The relative content of Radio-Hippuran in the rat erythrocytes found in the present work is of the same size as that of non-radioactive ortho-iodohippurate detected by Smith *et al.* (1945) *in vivo* in man and dog. The results of washing of the incubated erythrocytes indicated that no firm binding of the labelled substance to the red blood cells could be suspected. The nature of the uptake in the red blood cells might be a diffusion process or a very loose binding to any constituents in the erythrocytes.

It could also be concluded, from the results of the experiments of mixing Radio-Hippuran and oxyhemoglobin, that no considerable amounts of Radio-Hippuran were bound to this blood constituent. Indeed the normal oxyhemoglobin content *in vivo* was 16 times greater than that of the solution in this experiment. The results of the washing of the incubated erythrocytes contradict, however, that no firm binding may exist between Radio-Hippuran and oxyhemoglobin.

Summary

1. No or small amounts of the radioactive agent were bound to the serum proteins, when Radio-Hippuran was mixed with rat serum *in vitro* in concentrations of 0.04—10 mg per 100 ml serum.

2. When Radio-Hippuran was added to whole blood in amounts of 0.3 and 10 mg ortho-iodohippurate per 100 ml, the per cent of the given dose taken up by the red blood cells was equal irrespective of the added dose of ortho-iodohippurate. About 15 per cent was taken up during the first 5 minutes. Thereafter followed a slower uptake. The content of the radioactivity in the erythrocytes at 300 minutes after the mixing, amounted to about 25 per cent of that in whole blood. The Radio-Hippuran contained 9.1 per cent free radioactive iodide, which was shown to be promptly distributed among the plasma and the red blood cells in about equal concentration.

3. By washing the incubated red blood cells with physiologic saline a logarithmic diminishing of the radioactivity in the erythrocytes occurred. After the sixth washing less than 2 per cent remained in the erythrocytes, indicating that no firm binding occurred to any erythrocyte constituent.

4. No or small amounts of Radio-Hippuran combined with oxyhemoglobin as was shown by mixing a solution of oxyhemoglobin with tracer and carrier doses of radioactive sodium ortho-iodohippurate, and determing the binding by protein precipitation and electrophoresis.

CHAPTER VI

Animal experimental technique

Animals

Female albino rats weighing 160—240 g were kept in a uniform type of cages and had an adequate supply of milk, potatoes, meat, cereals, and vitamins water *ad libitum* The laboratory temperature was + 16 — + 20 C.

Anatomical notes on the rat kidneys

In the rat the right kidney lies at a more cranial level and more medially than the left. The liver partly covers the right kidney while the stomach lies partly over the left one. The kidneys were found to be 14—17 mm in length and 8—10 mm in breadth.

Anesthesia

In long time experiments the animals were given intraperitoneally a solution containing per ml 0 05 g Diminal (ethyl 1 methyl 1-butenyl malonyl-carbamide, Astra Ltd., Sweden) in a dosage of 0 05 g per kg body weight. An additional dosage of 0 008 g Diminal per kg body weight was necessary after 2—3 hours in order to maintain a constant depth of the anesthesia.

Some animals were anesthetized with ether for a few minutes when nephrectomy or administration of test substances was carried out. Afterwards they were allowed to move freely

Operation technique

Unilateral and bilateral nephrectomy were performed as follows after anesthetizing the animal with ether the abdomen was opened by a median incision and the kidneys were exposed. In animals on which radiorenograms were to be performed, the localization of the two kidneys as well as the mobility of the remaining kidney were examined. Double ligatures were tied around the renal vessels and around the ureter and the kidney then removed. A minimal bleeding occurred from the operation bed. The abdomen was closed with two series of silk sutures, one in the omentum parietale and musculature, and one in the skin.

To minimize any possible effect of the operation on the distribution and kinetics of Radio-Hippuran, the radioactivity experiments were performed on the day following the operation in bilaterally nephrectomized rats, and one-two days after the operation in unilaterally nephrectomized rats.

Injected solutions

a. The stock solutions or dilutions of the stock solutions of Radio-Hippuran containing fraction A in amounts of 90—96 per cent. The solutions were used within 20—25 days after the delivery date of the

stock solution. The radioactivity of the amounts injected varied between 10 and 20 μC.

b. The stock solutions of RISA with added albumin or dilutions of this solutions, were used within 20—25 days after the delivery date. The injected radioactivity amounted to 3—10 μC.

c. Preparation of a fraction A, containing 0.4—1.5 per cent of fraction B. The administered radioactivity was 3—10 μC. For preparation method, see *d*.

d. Preparation of a fraction B, containing 1.9—2.4 per cent of fraction A. The injected radioactivity amounted to 3—10 μC. The preparations of fractions A and B were obtained by chromatography in solvent I followed by elution of the fractions from the paper chromatogram with distilled water and concentration by freeze-drying.

e. Dilutions of carrier-free $Na^{131}I$ (Abbott). The radioactivity of the administered solutions was 3—15 μC.

f. Solutions of non-radioactive sodium ortho-iodohippurate (Hopkin & Williams Ltd., England). The original solution was diluted with varying amounts of physiologic saline. When the solutions *d* and *e* were administered, a dosage of 40 μg of non-radioactive sodium ortho-iodohippurate was simultaneously injected. In earlier experiments a dose of 20 mg non-radioactive sodium ortho-iodohippurate per 200 g body weight was administered.

g. Urine from normal rat injected with Radio-Hippuran.

h. Bile collected from a bilaterally nephrectomized rat injected with Radio-Hippuran.

Injection technique

The solutions were administered intravenously by means of an all-glass syringe of 1 ml total volume, graduated in one-hundredths of a milliliter. Needles (Record) no. 20 were used. The injection was made in an exposed femoral vein or in a sublingual vein. The volume of the administered solutions varied between 0.2 and 0.3 ml. The injection was completed within 2—3 seconds.

When two of the above mentioned substances were to be administered, they were injected simultaneously into the right and left femoral veins. The syringe containing the radioactive solution was weighed both before and after the injection. When performing external measurements the radioactivity of the contents of the syringe was counted both before and after injection under standard conditions (see Chap. III).

After opening of the abdomen in two animals, radioactive bile (solution *h*) was injected into the upper part of the small intestine. This organ was then ligated at both ends to prevent spreading of the radioactivity to other parts of the gastro-intestinal tract.

Error. For the error due to evaporation of the contents of the syringe, see Chap. III.

If the injection failed in any way (paravenous injection, leakage of the attached needle, etc.) the animal was excluded from further investigation.

Timing and duration of the experiments

An LKB-SC-31 interval timer with an accuracy of 0.01 minutes was started at

CHAPTER VI

Animal experimental technique

Animals

Female albino rats weighing 160—240 g were kept in a uniform type of cages and had an adequate supply of milk, potatoes, meat, cereals, and vitamins water *ad libitum* The laboratory temperature was + 16 — + 20 C.

Anatomical notes on the rat kidneys

In the rat the right kidney lies at a more cranial level and more medially than the left. The liver partly covers the right kidney while the stomach lies partly over the left one. The kidneys were found to be 14—17 mm in length and 8—10 mm in breadth.

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If the injection failed in any way (paravenous injection, leakage of the attached needle, etc.) the animal was excluded from further investigation.

Timing and duration of the experiments

An LKB-SC-31 interval timer with an accuracy of 0.01 minutes was started at

the beginning of the intravenous injection of the labelled substances.

Collection of bile and urine, and blood sampling were, as a rule, carried out for 300 minutes. The distribution studies were performed by removing organs and tissues at the following time intervals 1 4 10 30 100 and 300 minutes after administration of the test substance. The externally measured radioactivity was followed for 30—300 minutes.

Collection of urine

Several methods were tried in order to measure continuously the excreted radioactivity in urine of rats. The following technique was found to be the most suitable.

When the animal was sufficiently anesthetized, the abdomen was opened in the median line above the symphysis, and the fundus of the urinary bladder was incised. A polyethylene catheter (Portex no. 53 inner diam. 0.50 mm, outer diam. 0.90 mm, length 6 cm) with the cranial end somewhat widened was introduced through the opening of the urinary bladder and passed down through the urethra until the cranial end of the catheter was situated just above the beginning of the urethra. The catheter was fixed at the outer end of the urethra with a ligature. Another polyethylene catheter (Portex no. 47 inner diam. 0.20 mm, outer diam. 0.50 mm) with one end widened was introduced through the fundus with this widened end, and the catheter was fixed with a ligature around the fundus. This catheter was connected with a drop aggregate with physiologic saline. In this way it was possible to wash the urinary bladder with varying amounts of physiologic saline. The urine and the washing fluid ran out through the catheter in the urethra and were collected in plastic tubes. The drop velocity of the washing fluid was about 1 ml per minute during the first 20 minutes, and after this time, 0 05—0.2 ml per minute. The collection intervals were 1 2 5 10 and 20—60 minutes during the periods 0—10, 10—20 20—40 40—100 and 100—300 minutes respectively

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Collection of bile

Collection of bile was performed with the same technique as described by Meurman in 1960 after the animal was sufficiently anesthetized, the abdomen was opened in the median line and the common bile duct exposed. About 1 cm distal to the junction of the hepatic bile ducts, the common bile duct was ligated. Just above this ligature a polyethylene catheter (Portex no. 47) was introduced through a small incision in the common duct. After checking the free passage of bile flow the catheter was fixed with a ligature around the common bile duct containing the catheter The length of the catheter was adjusted to 7 cm. The abdomen was then closed by small clips. The bile was collected in plastic tubes in 10 to 60 minute periods.

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Blood sampling

Blood samples were obtained from the tail of the rat, which was incised tangentially with a razor blade. At the beginning of the experiment 0.05 ml, and at the end 0.10 ml, blood were drawn into a pipet. The time required to perform this procedure was usually less than 20 and 50 seconds respectively. The contents of the pipet were blown out into a plastic tube containing 2 ml distilled water. The pipet was carefully rinsed with the water in the plastic tube.

The results of these studies were given as the content of radioactivity of the whole blood volume in per cent of the injected radioactivity. The blood volume was indirectly estimated by weighing the animal, and was set at 7 per cent of the body weight as recommended by Wang and Hegsted (1949).

Error The volumes of the pipets used for blood sampling were found not to vary more than ± 0.1 per cent from the stated volumes. The errors due to pipetting were, on the average, ± 1 per cent of the mean.

Removal of organs

In the experiments performed to study the general distribution of the test substance, the following technique was used. The rat was usually anesthetized with Dipinal, in some cases with ether, then placed on its back and fixed with adhesive tape at its limbs on the operating table, which was kept at a temperature of 38° C. After administration of the test substance a ligature was applied around the outer end of the urethra. In the experiments lasting more than 30 minutes, this ligature was loosened and the urinary bladder emptied by easy compression at 100—200 minutes. After the thoracic cavity was opened at the end of the experiment, the animal was killed by strangulation of aorta ascendens and vena cava superior et inferior with a ligature at the root of the heart. In this way a prompt interruption of the blood circulation was obtained. After 2—3 minutes the ligature of the heart root was loosened, the heart and the lungs were removed and the blood from the sectioned vessels was allowed to run out into the thoracic cavity. As soon as possible the blood was drawn up into a pipet and blown out into a glass tube which was then sealed with parafilm. 4—6 ml blood were collected in this way. The abdomen was then opened, and two ligatures were applied around the kidney vessels and each ureter as near as possible to the hili of the kidneys. The ureters were divided between the ligatures, and the kidneys removed together with the upper end of the ureters. The urinary bladder with the two ureters and the urethra was taken out in one piece and was counted together with the collected urine. The liver was removed after ligation of the bile ducts

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and liver blood vessels. Its blood contents were emptied by careful squeezing. The total gastrointestinal tract was removed, and then divided as follows: stomach, small intestine, coecum, and colon. The contents of each of these parts were usually emptied by washing with 10 ml physiologic saline. The whole brain was taken out. Samples of skin and muscle were taken: skin from the back and muscle from the leg, which was not injected with the radioactive material. The surface of the removed organs was cleaned from blood before they were placed in glass tubes with 10 per cent NaOH. When the radioactivity in relation to weight was determined, the organ or tissue was wrapped up in a piece of parafilm which was weighed both with and without the organ in order to check the weight of the organ. The organ or tissue was then homogenized together with the parafilm.

In order to control that the total radioactivity found in the rat agreed with that of the given dose of ^{131}I the rest of the rat body was, in some cases, homogenized in 10 per cent NaOH with a total volume of 300 ml in a boiling water bath for an hour. With exception of the bones and teeth, a complete disintegration of the rat body occurred, resulting in a red-gray solution with a top layer of fat. After careful stirring of the solution two or three 12 ml samples were measured in the same way as the organs.

The measurements were presented as the radioactive content in per cent of the given dose, in some cases in per cent per gram tissue per 200 g body weight.

Errors. The error due to migration of radioactive substance out from organs and tissues *post mortem* was avoided by removal of the organs and tissues as soon as possible, usually within 5—10 minutes after killing of the animals.

The diminishing of the radioactivity of organs by evaporation was controlled by allowing a removed ^{131}I-containing organ, lung, to stay at room temperature and measuring the remaining radioactivity at different time intervals. After 3 hours the radioactivity of the organ had decreased by about 1.3 per cent, allowance being made for the decay of the isotope.

The loss of radioactivity upon homogenization of organs did not exceed 0.5 per cent.

Weight measurements

Organs, samples, and syringes were weighed on a Mettler semi micro balance, with an estimated precision of ± 0.00002 g.

Technique of external measurement of radioactivity

The anesthetized animal lying on its back had its limbs fixed by adhesive tape to an operating table which was heated by a lamp. External radioactivity registrations were performed simultaneously over two parts of the body by means of the apparatus for continuous external scintillation measurements described in Chapter III. One of the detectors was pointed to the animal from above and one detector from below. In some experiments the gamma ray spectrometer was joined to the apparatus for external measurements allowing only impulses with energies between 0.32 and 0.42 MeV to be registered.

The radioactivity of the syringe was counted immediately before and after the injection under standard conditions as described in Chapter III. The difference

in counts was regarded as the administered radioactivity

The time constant of the ratemeter was 0.5 second during the first 11.25—15 minutes after the injection, and after this time 2.5 seconds. The paper speed of the writer during these periods was 51 and 6.8 mm per minute respectively

The external measurements were carried out over three parts of the body principally different in respect to the distribution and kinetics of Radio-Hippuran. Thus the radioactivity was checked over each kidney representing the main excretory organ (renogram or radiorenogram) over urinary bladder (cystogram or radiocystogram) serving as accumulatory organ of the excreted radioactivity and over other parts of the body different in circulatory respect a central part (heart) a peripheral part (tail) and a medio-peripheral part (head) In some rats external scintillation measurements were performed over the liver (hepatogram or radiohepatogram) and the thyroid gland.

Radiorenogram

The external scintillation measurements over each kidney were carried out throughout the whole experimental series with the same equipment as that which was controlled with a Co^{60} standard (see Chap III) The detector was pointed to the kidney from below The distance from the outer perture of the collimator to the rat was 10 mm. From anatomical studies of the topography of the kidneys it was established that the left and right kidneys were most suitably exposed to the detectors if the outer apertures of the collimators were placed as follows The medial edge of the outer aperture of the collimator for the left kidney should be 5 mm to the left of the median line of the rat, and the cranial part of the opening should just touch the vertical projection plane of the ventral part of left costal margin. For the right kidney the medial edge of the collimator should be 3—5 mm to the right of the median line of the rat, and the center of the collimator should point 3—5 mm caudally to the right costal margin in the medioclavicular line. In nephrectomized rats the detectors were pointed to the place, where the kidneys were situated in laparotomy The duration of the external measurement depended upon how long it was possible to keep the animal in the same position, usually for 30—300 minutes. The radioactivity was registered over the intact kidney as well as over the place of the removed kidney

An original Radio-Hippuran renogram curve from a normal rat is shown in fig 13 A prompt rise of the radioactivity (A) occurred immediately after the injection of the labelled substance due to filling of the vascular bed with the radioactive material. This segment was followed by a secondary slower rise (B) reflecting the accumulation of Radio-Hippuran in the kidney Within 1—2 minutes a maximum of the radioactivity (M) was reached. Thereafter a drop of the radioactivity (C) was observed due to excretion of the tagged substance to the urinary bladder Since the radiorenogram curve did not reflect the changes of the radioactivity only in the kidney but also in the surrounding tissue and organs, attempts were made to calculate the body background. Thus radioactivity curves were also performed over the place of the removed kidney In normal rats the background radioactivity was determined at the different time intervals after injection of the radioactive

and liver blood vessels. Its blood contents were emptied by careful squeezing. The total gastrointestinal tract was removed, and then divided as follows: stomach, small intestine, coecum, and colon. The contents of each of these parts were usually emptied by washing with 10 ml physiologic saline. The whole brain was taken out. Samples of skin and muscle were taken: skin from the back and muscle from the leg which was not injected with the radioactive material. The surface of the removed organs was cleaned from blood before they were placed in glass tubes with 10 per cent NaOH. When the radioactivity in relation to weight was determined, the organ or tissue was wrapped up in a piece of parafilm, which was weighed both with and without the organ in order to check the weight of the organ. The organ or tissue was then homogenized together with the parafilm.

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The radioactivity of the syringe was counted immediately before and after the injection under standard conditions as described in Chapter III. The difference

in counts was regarded as the administered radioactivity

The time constant of the ratemeter was 0.5 second during the first 11.25—15 minutes after the injection, and after this time 2.5 seconds. The paper speed of the writer during these periods was 51 and 6.8 mm per minute respectively

The external measurements were carried out over three parts of the body principally different in respect to the distribution and kinetics of Radio-Hippuran. Thus the radioactivity was checked over each kidney representing the main excretory organ (renogram or radiorenogram) over urinary bladder (cystogram or radiocystogram) serving as accumulatory organ of the excreted radioactivity and over other parts of the body different in circulatory respect a central part (heart) a peripheral part (tail) and a medio-peripheral part (head) In some rats external scintillation measurements were performed over the liver (hepatogram or radiohepatogram) and the thyroid gland.

Radiorenogram

The external scintillation measurements over each kidney were carried out throughout the whole experimental series with the same equipment as that which was controlled with a Co^{60} standard (see Chap III) The detector was pointed to the kidney from below The distance from the outer aperture of the collimator to the rat was 10 mm. From anatomical studies of the topography of the kidneys it was established that the left and right kidneys were most suitably exposed to the detectors if the outer apertures of the collimators were placed as follows The medial edge of the outer aperture of the collimator for the left kidney should be 3 mm to the left of the median line of the rat, and the cranial part of the opening should just touch the vertical projection plane of the ventral part of left costal margin. For the right kidney the medial edge of the collimator should be 3—5 mm to the right of the median line of the rat, and the center of the collimator should point 3—5 mm caudally to the right costal margin in the medioclavicular line. In nephrectomized rats the detectors were pointed to the place, where the kidneys were situated in laparotomy The duration of the external measurement depended upon how long it was possible to keep the animal in the same position, usually for 30—300 minutes. The radioactivity was registered over the intact kidney as well as over the place of the removed kidney

An original Radio-Hippuran renogram curve from a normal rat is shown in fig 13 A prompt rise of the radioactivity (A) occurred immediately after the injection of the labelled substance due to filling of the vascular bed with the radioactive material. This segment was followed by a secondary slower rise (B) reflecting the accumulation of Radio-Hippuran in the kidney Within 1—2 minutes a maximum of the radioactivity (M) was reached. Thereafter a drop of the radioactivity (C) was observed due to excretion of the tagged substance to the urinary bladder Since the radiorenogram curve did not reflect the changes of the radioactivity only in the kidney but also in the surrounding tissue and organs, attempts were made to calculate the body background. Thus radioactivity curves were also performed over the place of the removed kidney In normal rats the background radioactivity was determined at the different time intervals after injection of the radioactive

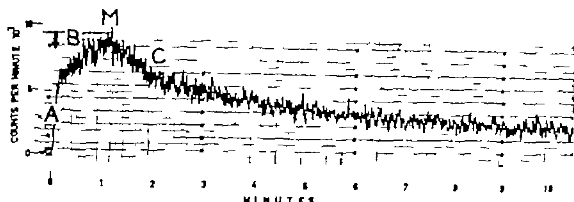


Fig 13. Original radiorenogram curve from a normal rat after intravenous injection of Radio-Hippuran. A — Initial phase. B — accumulation phase. M — maximum point. C — excretion phase. Arrow marks the time of injection.

material after which the animals were killed by strangulation of the heart. The abdomen was opened and the kidneys were removed after ligation of the renal vessels and the ureters. The organs of the abdomen were then moved back again to their normal position. The radioactivity over the kidney area was measured externally before and after the excision of the kidney.

The radioactivity curve was plotted on semilogarithmic paper after correlation to an administered radioactivity giving 100 000 counts per minute in the apparatus where the syringe was counted.

Radiocystogram

External measurements were carried out with the scintillation detector pointed to the urinary bladder from above. The distance between the collimator and the skin of the rat was about 5 mm. The collimator was placed in the median line of the rat with the caudal edge of the aperture just at the symphysis. The external measurements were usually made during 30—150 minutes.

The shape of Radio-Hippuran cystogram curve from a normal rat is seen in fig 14. An initial slight rise (A) followed by a diminishing of the radioactivity (B) is seen during the first one-two minutes and is referred to as the body background. Thereafter a secondary rise (C) followed, the rate of which was initially high, but decreased continuously. The segment C represents the accumulation of the radioactivity in the urinary bladder (Magnusson, 1957).

The "net" cystogram curve was constructed by subtraction of an extrapolated background curve from the original radiocystogram curve (fig 14).

After 30—150 minutes the experiments were finished. The amount of ^{131}I excreted was estimated in two ways. Some animals were killed, the urinary bladders removed and their radioactivity estimated. From other animals samples of urine were obtained by careful pressing on the urinary bladder tract. The decrease (*b*) of the radioactivity level over the bladder was set equal to the per cent of given dose recovered in the urine sample. From this value the real content of ^{131}I in the urinary bladder (*a*) was determined. The

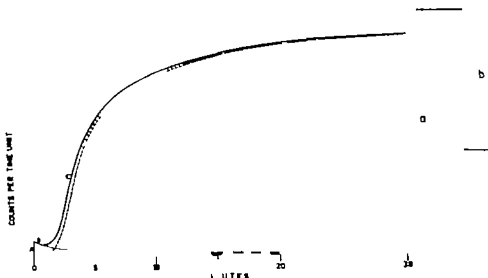


Fig 14 Radiocystogram curv from normal rat after intravenous injection of Radio-Hippuran. A and B represent the body background *a*. the radioactivity content in blood and tissue surrounding the urinary bladder C = rising phase depending on the accumulation of Radio-Hippuran in the urinary bladder When the slowly decreasing phase (B) representing the body background, is subtracted from the radiocystogram curve the resulting curve will be the urinary accumulation curve, which is seen just under the radiocystogram curve. The level of the radio-cystogram curve after long time is denoted by *a*. The decrease of the radioactivity level over the urinary bladder after the expression of quantity of urine is denoted by *b*

amount of radioactivity excreted into urine at different time intervals could then be calculated.

Radiohepatogram

The detector was pointed from above to the right lateral lobe of the liver When the right kidney remained the detector was directed somewhat cranially in order to void influence from the radioactivity in this organ

External measurements over the thyroid gland

The detector was directed from above with the caudal edge of the outer collimator opening just above *manubrium stern*

External measurements over other parts of the body

A. The measurements over the heart were made with detector pointed to the upper part of the chest from above with a distance of about 5 mm between the collimator and the skin of the rat.

B. Radioactivity curves over the head were obtained with the detector from above, the collimator about 5 mm from the rat and the cranial edge of the aper ture t the nose-tip of the rat.

C. External scintillation curves of the tail of the rat were obtained with nearly the whole tail hanging down in the hole of the inverted collimator.

One of the solutions mentioned in *a*, *c* and *d* was injected. In connection with

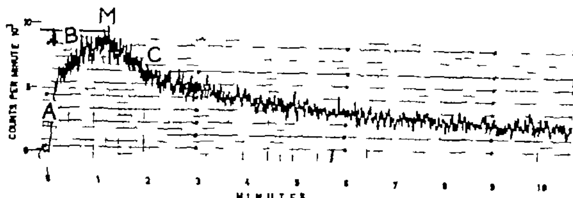


Fig 13. Original radiorenogram curve from a normal rat after intravenous injection of Radio-Hippuran. A = initial phase. B = accumulation phase. M = maximum point. C = excretion phase. Arrow marks the time of injection.

material after which the animals were killed by strangulation of the heart. The abdomen was opened and the kidneys were removed after ligation of the renal vessels and the ureters. The organs of the abdomen were then moved back again to their normal position. The radioactivity over the kidney area was measured externally before and after the excision of the kidney

The radioactivity curve was plotted on semilogarithmic paper after correlation to an administered radioactivity giving 100 000 counts per minute in the apparatus where the syringe was counted

Radiocystogram

External measurements were carried out with the scintillation detector pointed to the urinary bladder from above. The distance between the collimator and the skin of the rat was about 5 mm. The collimator was placed in the median line of the rat with the caudal edge of the aperture just at the symphysis. The external measurements were usually made during 30—150 minutes.

The shape of Radio-Hippuran cystogram curve from a normal rat is seen in fig 14 An initial slight rise (A) followed by a diminishing of the radioactivity (B) is seen during the first one two minutes and is referred to as the body background. Thereafter a secondary rise (C) followed, the rate of which was initially high, but decreased continuously The segment C represents the accumulation of the radioactivity in the urinary bladder (Magnusson, 1957)

The "net" cystogram curve was constructed by subtraction of an extrapolated background curve from the original radiocystogram curve (fig 14)

After 30—150 minutes the experiments were finished The amount of ^{131}I excreted was estimated in two ways. Some animals were killed, the urinary bladders removed and their radioactivity estimated. From other animals samples of urine were obtained by careful pressing on the urinary bladder tract. The decrease (b) of the radioactivity level over the bladder was set equal to the per cent of given dose recovered in the urine sample. From this value the real content of ^{131}I in the urinary bladder (a) was determined The

CHAPTER VII

Behaviour of Radio-Hippuran in normal rats

Part I

General distribution

Four groups of rats were injected with Radio-Hippuran, fraction A, fraction B, and $Na^{131}I$ and the radioactivity in organs was measured at various time intervals.

Radio-Hippuran

The radioactivity in organs which were assumed to accumulate, excrete, or metabolize the test substance was measured in four anesthetized animals, and other organs and tissues in two anesthetized rats, killed at each of the following time intervals 1 4 10, 30, 100, and 300 minutes. In order to check whether or not anesthesia had an effect on the distribution of the injected material, one non-anesthetized animal was examined at 100 and another at 300 minutes.

Results

The results of the distribution studies of Radio-Hippuran are shown in tables III and IV.

The main part of the radioactivity was eliminated through the kidneys into the urine w thin 30 minutes. A small portion was excreted into the stomach and the small intestine. A significant uptake of radioactivity ($P < 0.01$) was also observed in the thyroid gland. The radioactivity in most of the organs and tissues decreased at the same rate as that of the blood radioactivity. During the first 100 minutes the radioactivity diminished in the kidneys at faster rate than in the blood. The radioactivity of the total gastrointestinal tract remained at a rather constant level from 4 minutes on in spite of the fact that the radioactivity in the contents of the stomach and the small intestine increased.

The distribution of Radio-Hippuran in the non-anesthetized rats agreed rather well with that of the other animals.

Fraction A

The distribution of this fraction was studied in nine rats. Three animals were killed at each of the following time intervals 1 30 and 300 minutes after the injection.

Results

The amounts of the radioactivity in some pertinent organs are presented in

the external measurements after administration of one of the above mentioned solutions, the external radioactivity courses were followed with the rat and detector *in the same positions after injection of* solution *b* (RISA) as a rule, at the end of the experiment. In some cases the test substances were injected in inverted order The amount of radioactivity administered was determined by counting the syringe both before and after injection.

3—6 minutes after the injection of RISA the radioactivity over the three examined parts of the body lay at a constant level indicating complete mixing in the whole blood volume. This amplitude was denoted by 100 per cent. The amplitude of the Radio-Hippuran activity curve was correlated to the administered radioactivity of RISA. The values of the ratio of the amplitudes of Radio-Hippuran and of RISA were then plotted in a semilogarithmic system.

Errors

To get representative radioactivity curves when performing the radiorenograms and the radiocystograms it is important that the whole organ is exposed to the detector This was also controlled When the renogram was carried out about half a minute after the injection the animal was moved some millimeters cranially and caudally in relation to the collimator and was then moved back to its original position. As a rule the maximal radioactivity was registered at the original position of the animal, and the original position of the detector failed only in a few cases to give the highest numbers of counts.

In the external measurements over the urinary bladder 2—3 minutes after the injection, the detector was moved some millimeters cranially and caudally to determine the point where the maximal radioactivity was registered. The external measurement was then continued over this point.

For the reproducibility of the counter which was used in counting the syringe, and of the apparatus for performing external measurement under standard conditions, see Chapter III

Table IV Comparison of radioactivity content in various organs of normal rats, 1 30, and 300 minutes after intravenous injection of Radio-Hippuran and of fraction A. The values show the percentage of the dose administered and standard deviations.

Organs	1 minute		30 minutes		300 minutes	
	Radio-Hippuran	Fract. A	Radio-Hippuran	Fract. A	Radio-Hippuran	Fract. A
[illegible]	[illegible]	14.64 ± 2.15	0.49 ± 0.15	0.58 ± 0.12	0.03 ± 0.003	0.014 ± 0.002
[illegible]	[illegible]	[illegible]	0.39 ± 0.26	0.64 ± 0.23	0.04 ± 0.004	0.014 ± 0.009
[illegible]	[illegible]	[illegible]	88.39 ± 4.43	93.81 ± 2.70	91.92 ± 3.94	94.20 ± 2.10
[illegible]	[illegible]	[illegible]	1.72 ± 0.14	1.32 ± 0.16	0.49 ± 0.07	0.15 ± 0.02
[illegible]	[illegible]	[illegible]	0.65 ± 0.35	0.26 ± 0.19	1.65 ± 0.58	1.11 ± 0.21
[illegible]	[illegible]	[illegible]	0.60 ± 0.06	0.52 ± 0.13	0.15 ± 0.02	0.07 ± 0.01
Stomach	[illegible]	[illegible]	1.48 ± 0.43	0.82 ± 0.40	1.90 ± 0.38	0.38 ± 0.07
Small intestine	3.06 ± 0.62	2.84 ± 0.07	1.46 ± 0.21	0.96 ± 0.10	1.23 ± 0.26	0.16 ± 0.05
Coecum	0.70 ± 0.05	0.80 ± 0.15	0.14 ± 0.03	0.08 ± 0.04	0.09 ± 0.05	0.03 ± 0.01
Colon	0.85 ± 0.09	0.94 ± 0.20	0.13 ± 0.03	0.09 ± 0.01	0.06 ± 0.02	0.14 ± 0.17
Total gastrointestinal tract	5.56 ± 0.60	5.20 ± 0.20	3.13 ± 0.55	*1.93 ± 0.59*	3.39 ± *0.40*	*0.97* ± 0.22

table IV together with the results of the corresponding distribution of Radio-Hippuran.

On the whole the two substances behaved similarly. Minor differences in their distribution were, however found. Thus, at one minute the liver contained significantly more radioactivity when fraction A was given. At 30 minutes the blood and the small intestine contained more radioactivity after injection of Radio-Hippuran. Significantly lower radioactive contents were found in the kidneys, liver stomach, small intestine and the total gastrointestinal tract at 300 minutes after the administration of fraction A.

Fraction B and $^{131}I^-$

A group of si normal rats was injected with fraction B. Another group of six rats was injected with $Na^{131}I$. Non-radioactive sodium ortho-iodohippurate in a dose of 40 μg was simultaneously administered to the two groups. The radioactivity in some pertinent organs of two rats was determined at each of the following time intervals 1 30 and 300 minutes after the administration of the test substances.

Results

The radioactivity of the organs examined is found in table V

On the whole the distribution of fraction B and $^{131}I^-$ coincided. A tendency to accumulate more radioactivity in the thyroid gland and less in the blood was noticed when $^{131}I^-$ was given.

Discussion

The results of the distribution studies performed after injection of Radio-Hippuran are in rather good accordance with those found by zum Winkel and de Maria (1961) who measured the radioactivity in the kidneys, liver and blood at 10 30, and 150—200 minutes, and in urine, thyroid gland, and the different parts of the gastrointestinal tract at 150—300 minutes, after administration of Radio-Hippuran (Abbott) into rats. No other elimination or uptake of Radio-Hippuran than into kidneys, thyroid gland, and gastrointestinal tract has been detected.

From the results of the distribution studies of *fraction B, shown in table V* the radioactivity of this component in differ

Table III Content of radioactivity in various organs of normal rats, 1 4 10 30 100 and 300 minutes after intravenous administration of Radio-Hippuran. The values show the percentage of the dose administered and the standard deviations.

Organs	1 minute	4 minutes	10 minutes	30 minutes	100 minutes		300 minutes	
						Not anesthetized rat		Not anesthetized rat
Kidney left	18.82±4.00	5.41±1.10	1.86±0.39	0.49±0.15	0.07±0.03	0.04	0.03±0.003	0.03
Kidney right	17.65±1.40	5.32±1.10	2.61±1.40	0.59±0.26	0.07±0.02	0.04	0.04±0.004	0.03
Urinary bladder	2.84±1.10	48.48±3.35	72.03±9.90	88.39±4.43	91.72±2.09	—	91.92±3.94	—
Blood	23.66±2.00	11.41±1.17	5.03±0.62	1.72±0.14	0.76±0.18	0.05	0.49±0.07	0.36
Thyroid gland	0.23±0.19	0.40±0.29	0.41±0.19	0.63±0.55	0.70±0.30	1.30	1.66±0.58	3.76
Liver	8.57±0.84	4.07±0.90	2.16±0.11	0.60±0.06	0.24±0.03	0.19	0.15±0.02	0.09
Stomach wall	0.57±0.05	0.35±0.01	0.30±0.11	0.38±0.02	0.25±0.07		0.29±0.12	0.07
» contents	0.17±0.09	0.35±0.17	0.64±0.24	1.11±0.44	1.22±0.39	1.39	1.61±0.48	0.48
Small intestine wall	2.39±0.55	1.30±0.19	1.23±0.24	0.65±0.06	0.57±0.18		0.31±0.12	0.20
» » contents	0.67±0.29	0.69±0.25	0.84±0.23	0.81±0.22	1.15±0.37	1.94	0.92±0.27	1.35
Coecum wall	0.51±0.04	0.24±0.05	0.20±0.16	0.06±0.02	0.03±0.01		0.02±0.007	0.08
» contents	0.19±0.02	0.13±0.08	0.08±0.04	0.09±0.02	0.09±0.03	0.07	0.07±0.04	0.71
Colon wall	0.71±0.10	0.39±0.13	0.22±0.07	0.10±0.03	0.04±0.01		0.03±0.01	0.06
» contents	0.15±0.08	0.08±0.04	0.06±0.03	0.03±0.01	0.04±0.03	0.10	0.03±0.02	1.21
Total gastrointestinal tract	5.36±0.60	3.53±0.21	3.56±0.79	3.22±0.55	3.38±0.60	3.51	3.29±0.38	4.17
Spleen	0.27	0.12	0.06	0.03	0.01		0.01	
	0.21	0.14	0.07	0.03	0.01		0.04	
Gl. parotis	0.21	0.13	0.07	0.03	0.01		0.01	
	0.23	0.12	0.05	0.02	0.01			
Adrenal glands	0.036	0.023	0.012	0.003	0.003		0.002	
	0.030	0.020	0.017	0.006	0.006		0.001	
Lungs	0.69	0.39	0.28	0.10	0.04		0.02	
	0.79	0.42	0.37	0.10	0.04		0.02	
Uterus	0.668	0.254	0.163	0.111	0.050		0.004	
	0.555	0.342	0.272	0.213	0.326		0.013	
Ovaries	0.065	0.042	0.043	0.012	0.003		0.003	
	0.065	0.032	0.040	0.008	0.004		0.008	
Thymus	0.100	0.060	0.033	0.010	0.007		0.002	
	0.108	0.075	0.035	0.014	0.008		0.005	
Skin (per gram)	0.25	0.28	0.17	0.06	0.03		0.02	
	0.39	0.53	0.06	0.10	0.04		0.02	
Skeletal muscle (per gram)	0.19	0.14	0.08	0.03	0.02		0.01	
	0.12	0.18	0.23	0.04	0.03		0.01	
Pancreas (per gram)	0.53	0.33	0.11	0.07	0.03		0.01	
	0.63	0.19	0.17	0.08	0.04		0.06	
Brain (per gram)	0.062	0.034	0.042	0.009	0.010		0.004	
		0.034	0.050	0.016	0.004		0.005	

Table II. Comparison of radioactivity content in various organs of normal rats, 1, 30, and 300 minutes after intravenous injection of Radio-Hippuran and of fraction A. The values show the percentage of the dose administered and standard deviations.

Organs	1 minute		30 minutes		300 minutes	
	Radio-Hippuran	Fract. A	Radio-Hippuran	Fract. A	Radio-Hippuran	Fract. A
Kidney left	18.62 ± 4.00	14.64 ± 2.15	0.49 ± 0.15	0.58 ± 0.12	0.03 ± 0.003	0.014 ± 0.002
Kidney right	17.65 ± 1.40	14.48 ± 1.77	0.59 ± 0.28	0.64 ± 0.23	0.04 ± 0.004	0.014 ± 0.009
Urinary bladder	2.84 ± 1.10	5.99 ± 5.00	88.59 ± 4.43	93.81 ± 2.70	91.92 ± 3.94	94.20 ± 2.10
Blood	23.66 ± 2.00	20.86 ± 1.55	1.72 ± 0.14	1.32 ± 0.16	0.49 ± 0.07	0.15 ± 0.02
Thyroid gland	0.23 ± 0.19	0.09 ± 0.01	0.65 ± 0.55	0.26 ± 0.19	1.65 ± 0.58	1.11 ± 0.21
Liver	8.57 ± 0.84	10.57 ± 0.32	0.60 ± 0.06	0.52 ± 0.13	0.15 ± 0.02	0.07 ± 0.01
Stomach	0.76 ± 0.06	0.62 ± 0.04	1.48 ± 0.43	0.82 ± 0.40	1.90 ± 0.58	0.38 ± 0.07
Small intestine	3.06 ± 0.62	2.84 ± 0.07	1.46 ± 0.21	0.96 ± 0.10	1.23 ± 0.26	0.16 ± 0.05
Coecum	0.70 ± 0.05	0.80 ± 0.15	0.14 ± 0.03	0.08 ± 0.04	0.09 ± 0.05	0.03 ± 0.01
Colon	0.85 ± 0.09	0.94 ± 0.20	0.13 ± 0.03	0.09 ± 0.01	0.06 ± 0.02	0.14 ± 0.17
Total gastrointestinal tract	3.56 ± 0.60	5.20 ± 0.20	3.13 ± 0.55	1.95 ± 0.39	3.39 ± 0.40	0.97 ± 0.22

table IV together with the results of the corresponding distribution of Radio-Hippuran.

On the whole the two substances behaved similarly. Minor differences in their distribution were, however, found. Thus, at one minute the liver contained significantly more radioactivity when fraction A was given. At 30 minutes the blood and the small intestine contained more radioactivity after injection of Radio-Hippuran. Significantly lower radioactive contents were found in the kidneys, liver, stomach, small intestine, and the total gastrointestinal tract at 300 minutes after the administration of fraction A.

Fraction B and ^{131}I

A group of six normal rats was injected with fraction B. Another group of six rats was injected with Na ^{131}I. Non-radioactive sodium ortho-iodohippurate in a dose of 40 µg was simultaneously administered to the two groups. The radioactivity in some pertinent organs of two rats was determined at each of the following time intervals: 1, 30 and 300 minutes after the administration of the test substances.

Results

The radioactivity of the organs examined is found in table V.

On the whole the distribution of fraction B and ^{131}I$^-$ coincided. A tendency to accumulate more radioactivity in the thyroid gland and less in the blood was noticed when ^{131}I$^-$ was given.

Discussion

The results of the distribution studies performed after injection of Radio-Hippuran are in rather good accordance with those found by zum Winkel and de Maria (1961) who measured the radioactivity in the kidneys, liver and blood at 10, 30, and 150—200 minutes, and in urine, thyroid gland, and the different parts of the gastrointestinal tract at 150—300 minutes, after administration of Radio-Hippuran (Abbott) into rats. No other elimination or uptake of Radio-Hippuran than into kidneys, thyroid gland, and gastrointestinal tract has been detected.

From the results of the distribution studies of fraction B, shown in table V, the radioactivity of this component in differ

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						Not anesthetized rat		Not anesthetized rat
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Kidney right	17 65 ± 1 40	5.52 ± 1 10	2.61 ± 1 40	0.59 ± 0.26	0.07 ± 0 02	0 04	0 04 ± 0.004	0.03
Urinary bladder	2 84 ± 1 10	48.48 ± 3.35	72 03 ± 9.90	88 39 ± 4 43	91 72 ± 2.09	—	91.92 ± 3.94	—
Blood	23 66 ± 2 00	11 41 ± 1 17	5 03 ± 0 62	1 72 ± 0 14	0 76 ± 0.18	0 05	0.49 ± 0.07	0 36
Thyroid gland	0.23 ± 0 19	0 40 ± 0.29	0 41 ± 0 19	0 65 ± 0.55	0 70 ± 0.30	1.30	1 66 ± 0.58	3 76
Liver	8.57 ± 0 84	4 07 ± 0 90	2 16 ± 0 11	0 60 ± 0 06	0.24 ± 0 03	0.19	0 15 ± 0.02	0.09
Stomach wall	0.57 ± 0.05	0.33 ± 0 01	0 30 ± 0 11	0 38 ± 0 02	0.23 ± 0 07		0.29 ± 0 12	0 07
» contents	0 17 ± 0 09	0 35 ± 0 17	0 64 ± 0.24	1 11 ± 0.44	1 22 ± 0.39	1 39	1 61 ± 0 48	0.48
Small intestine wall	2 39 ± 0.55	1.30 ± 0 19	1.23 ± 0.24	0 65 ± 0 06	0.57 ± 0 18		0.31 ± 0 12	0.20
» » contents	0 67 ± 0.29	0 69 ± 0.25	0.84 ± 0.23	0.81 ± 0.22	1 15 ± 0.37	1.94	0 92 ± 0.27	1.35
Coecum wall	0.51 ± 0 04	0.24 ± 0 05	0.20 ± 0 16	0 06 ± 0 02	0 03 ± 0 01		0 02 ± 0.007	0 08
» contents	0 19 ± 0 02	0 13 ± 0 08	0 08 ± 0 04	0.09 ± 0 02	0 09 ± 0 03	0.07	0 07 ± 0.04	0 71
Colon wall	0 71 ± 0 10	0 39 ± 0 13	0.22 ± 0 07	0 10 ± 0 03	0 04 ± 0 01		0 03 ± 0 01	0 06
» contents	0 15 ± 0 08	0 08 ± 0 04	0 06 ± 0 03	0.03 ± 0.01	0 04 ± 0 03	0 10	0 03 ± 0 02	1.21
Total gastrointestinal tract	5.36 ± 0 60	3.53 ± 0.21	3.56 ± 0.79	3.22 ± 0.55	3 38 ± 0 60	3.51	3.29 ± 0 38	4 17
Spleen	0.27	0 12	0 06	0 03	0 01		0.01	
	0.21	0 14	0 07	0 03	0 01		0.04	
Gl. parotis	0.21	0 13	0 07	0 03	0 01		0 01	
	0.23	0.12	0 05	0 02	0 01			
Adrenal glands	0.036	0 023	0 012	0.003	0 003		0 002	
	0 030	0 020	0 017	0 006	0 006		0 001	
Lungs	0 69	0.39	0.28	0 10	0 04		0 02	
	0 79	0 42	0 37	0 10	0 04		0 02	
Uterus	0.668	0.254	0 163	0 111	0 050		0 004	
	0.555	0 342	0 272	0.213	0.326		0 013	
Ovaries	0 065	0 042	0 043	0 012	0 003		0.003	
	0 065	0.032	0.040	0 008	0.004		0.008	
Thymus	0 100	0 060	0 033	0 010	0 007		0 002	
	0 108	0 073	0 035	0 014	0 008		0 005	
Skin (per gram)	0.25	0.28	0.17	0 06	0 03		0 02	
	0.39	0.33	0 06	0 10	0 04		0 02	
Skeletal muscle (per gram)	0 19	0 14	0 08	0 03	0.02		0 01	
	0.12	0 18	0.23	0 04	0 03		0 01	
Pancreas (per gram)	0.53	0 33	0 11	0 07	0 03		0 01	
	0.63	0 19	0 17	0 08	0 04		0.06	
Brain (per gram)	0 062	0 034	0 042	0 009	0 010		0.004	
		0 034	0.030	0.016	0.004		0 005	

were excreted into the stomach and the small intestine. A small but significant uptake of radioactivity occurred in the thyroid gland.

2. Fraction A was found to be more nephroattractive than the commercial preparation of Radio-Hippuran. Thus, more radioactivity was excreted into the urine, and less remained in the blood and was recovered in the kidneys, liver stomach, and small intestine.

3. The distribution of fraction B followed very closely that of radioactive iodide.

Part 2

Behaviour of Radio-Hippuran in blood

In this part, the principal purpose was to study the kinetics of the disappearance of Radio-Hippuran from blood by means of blood sample measurements and external radioactivity tracings over different regions of the body Furthermore, attempt was made to interpret the biologic importance of the results found *in vitro* as to the migration of Radio-Hippuran into the red blood cells, and its non-affinity to the serum proteins.

Measurements by blood sampling

Different groups of animals were followed for 200—300 minutes by recording the radioactivity in tail blood samples at intervals.

Seven rats were injected with Radio-Hippuran.

Two rats were injected with fraction A.

Two rats were injected with fraction B.

Result

The blood curves are graphically represented in fig. 15 A. The radioactivity values are expressed as percentage of the given dose. 100 per cent of given dose was assumed to exist in the blood at zero time. For comparison, blood radioactivity values according to the distribution studies (thoracic blood sampling) in Part 1 of this chapter are given in the figure.

Radio-Hippuran. The blood radioactivity according to tail blood measurements decreased rapidly within the first one—two minutes to about 20 per cent and continued to fall at an ever diminishing rate for another 60—80 minutes. After this time the radioactivity decreased at an exponential rate. When compared with the values of the thoracic blood samples somewhat higher values were consistently obtained from the tail blood. The same exponential disappearance rate was, however recorded in both curves between 100 and 300 minutes.

Fraction A. Principally the same shape of the disappearance curve resulted as when Radio-Hippuran was injected, but lower concentration of the radioactivity was consistently observed.

Fraction B. After decrease to about 20 per cent during the first one—two minutes a slope of very slowly falling character was noticed.

The uptake of Radio-Hippuran in red blood cells *in vivo*

In one rat injected with Radio-Hippuran, blood samples in amounts of 0.005

Table V Comparison of radioactivity content in various organs of normal rats, 1 30 and 300 minutes after intravenous injection of fraction B and of Na L The values show the percentage of the dose administered.

	1 minute		30 minutes		300 minutes	
	Fract. B	$^{131}I^-$	Fract. B	I^-	Fract. B	^{131}I
Kidney left	1.38 1 78	1 46 1.25	0 49 0.45	0.45 0.41	0.19 0.27	0 18 0.16
Kidney right	1.50 1.81	1 43 1 12	0.49 0.53	0.43 0.42	0.16 0.26	0.16 0.16
Urinary bladder	1 18 1 04	0.95 1.50	9.83 5 61	9 43 6.14	41 17 18.07	30.56 18.09
Blood	29.82 30.28	35 14 32.68	12 75 12.57	10 76 9.56	4 07 5 60	3.82 3.31
Thyroid gland	1.88 2.23	2.37 2.70	3.68 6.58	9 10 7 01	18.78 31 45	31 78 43.80
Liver	6.60 8.30	8.57 6.05	2.62 2.87	2.27 1.88	0.95 1 44	0.83 0.99
Stomach	4.31 2.83	2.79 4 12	16.00 16 66	20.88 14.95	8.47 13 67	13.10 12.34
Small intestine	8.12 7 48	9 19 7 66	7 03 4 63	7.37 7.50	2.56 4.23	7 09 4 46
Coecum	0.92 0.90	1.30 1 04	0.91 0.71	0.45 0.78	0 48 0 67	0.25 0.20
Colon	1 12 1 82	1 75 1 46	0 77 0.56	0.82 0 79	0.36 0.37	0.32 0.29
Total gastrointestinal tract	14 47 13 03	15 04 14.28	24 72 22.56	29.52 24 03	11.88 18.93	20.76 17.30

ent organs when Radio-Hippuran was injected can be calculated. The differences between these calculated values and those found after injection of Radio-Hippuran (tables III and IV) should give the amounts of radioactivity of fraction A (table IV) Such calculated values of fraction A and those found in the distribution study with a pure fraction A agreed in *most of the organs. In the thyroid gland* at 300 minutes, however these values did not agree. Less radioactivity than expected from the calculation was recovered in the thyroid gland after injection of Radio-Hippuran. No explanation of this fact can be given at present.

The amounts of radioactivity recovered in the thyroid gland were, however too small to have an influence on the general distribution and kinetics of Radio-Hippuran.

The biological behaviour of fraction B supports the radiochemical examinations that this component of Radio-Hippuran is identical to $^{131}I^-$

Summary

1 Distribution studies of Radio-Hippuran *in rats have shown that* about 88 per cent of the radioactivity was eliminated through the kidneys within 30 minutes. Small amounts of the radioactivity



Fig 16 Time-radioactivity curves of whole blood and red blood cells from normal rat after intravenous injection of Radio-Hippuran.

ml were collected in an heparinized poly ethylene catheter (Portex No. 53) which was handled in the same way as in the *in vitro* experiments (Chap. V)

Results

The radioactivity concentration of whole blood as well as that of the red blood cells is presented in fig 16. On the whole, the radioactivity of the erythrocytes diminished at the same rate as that of the whole blood. At the beginning about 25 per cent, and at the end of the experiment about 40 per cent of the total radioactivity in blood was recovered in the red blood cells.

The binding of Radio-Hippuran to serum proteins *in vivo*

One group of rats was injected with a tracer dose of Radio-Hippuran, and another group with non-radioactive sodium ortho-iodohippurate in amounts of 20 mg, with simultaneous tracer dose of the radioactive test substance. The rats were killed 1—. minutes after the injection and serum was examined principally as in the *in vitro* experiments (Chap. V)

1 Protein precipitation was performed as *in vitro*

2. Dialysis. 1 ml of serum from each group of animals was dialyzed against 100 ml isotonic phosphate buffer at pH 7 4 and +4 C. After 48 hours the buffer solution was replaced with fresh solution. The bags and 1 ml of the buffers were counted at 20, 48, and 72 hours after the beginning of the dialysis.

3. Ultrafiltration. Sera in amounts of 0.5—1 ml, stored in collodion tubings with surrounding isotonic phosphate buffer solution of pH 7 4 and +4 C, were sucked at a pressure of 40 mm Hg for 20 hours. At 15 hours after the beginning of suction, the buffer solution was replaced with fresh solution, and 0.5—1 ml isotonic neutral phosphate buffer was added to the contents of the tubings. The radioactivity of the tubings was measured before and after 15 and 20 hours from the beginning of the suction.

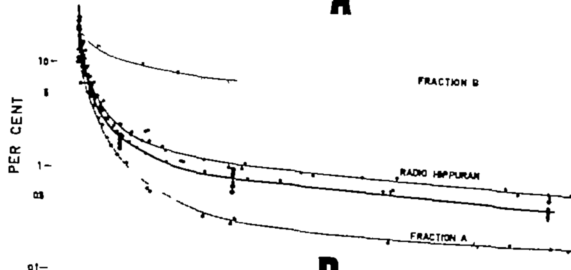
4 Electrophoresis. This procedure was the same as that described in the *in vitro* experiments.

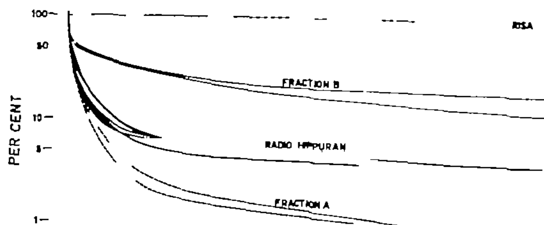
Results

1 Protein precipitation. The radioactivity of the precipitate amounted to 0.9—1 1 per cent of the radioactivity in the serum samples in the tracer experiment, and 0.4—0.6 per cent in the carrier experiment.

2. *Dialysis*. At 20 and 48 hours the ratios of serum to buffer [1] I were, in the tracer experiment, 8.2 and 5.6 respectively and in the carrier experiment, 5 2 and 5.0 respectively The radioactivity remaining in the bags at 72 *hours amounted* to 1 4 per cent in the tracer experiment and to 1 1 per cent in the carrier experiment.

3. Ultrafiltration. The radioactivity of the tubings at 15 and 20 hours in the tracer test was 6.1 and 3.9 *per cent* respectively *and 4.0* and 2.6 per cent in the carrier experiment.





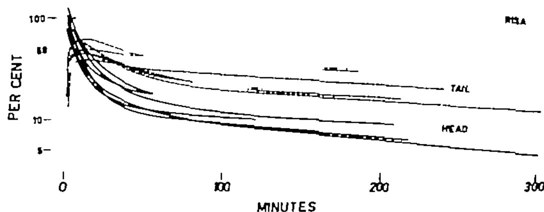


Fig 15 Time-concentration curves of radioactivity in blood (A) over the heart (B) and over the head and tail (C) In A, after injection of Radio-Hippuran (tail blood sample = small dots and thin line, thoracic blood = big dots and heavy line) fraction A and fraction B. In B, after injection of Radio-Hippuran, fraction A and fraction B. In C, after injection of Radio-Hippuran. In B and C the radioactivity level of RISA is set at 100 per cent.

nential processes (Teorell, 1937 Veall and Vetter 1958, Odeblad *et al.* 1959) If an accurate analysis in this respect is to be made, the measurements must be continued for a sufficiently long time so that a final exponential phase is reached. By graphical subtraction of this final phase from the original curve, other components may be revealed. Such graphical analysis of the blood sample curves as well as of the external measurements over the heart, head, and tail, has been performed.

Graphical plotting

In the blood sample curves, after injection of each of the three compounds, a final exponential component (phase 3) was obtained When this was subtracted from the original tracing, a curve was acquired, the slope of which decreased exponentially (phase 2) By applying the graphical subtracting procedure on this new curve, another component (phase 1) was detected in the curves obtained after injection of Radio-Hippuran and fraction A

In the external measurements, a final exponential phase could be shown during 50—500 minutes. By the graphical evaluation just described a second and third disappearance phase could be revealed in the curves recorded over the heart and head The tail radioactivity curve could be resolved into two decreasing phases and one uptake phase.

The exponential constants (expressed as fractions removed per minute) were graphically estimated and denoted k_1 k_2, and k_3, respectively The corresponding intercepts on the ordinate axis are denoted a_1 a_2, and a_3. The k and a-values are presented in table VI

Quantitative aspects

The values of the disappearance constant k_3, calculated from the curves obtained by blood sampling as well as external measurements after injection of Radio-Hippuran, did not differ significantly from each other Similarly the k_2 constants of these curves agreed fairly well, as did also the k_1 constants, with the exception of k_1 of the tail radioactivity curve. The parameters of the various phases of the external tracings showed higher values than the corresponding ones of the blood sample curve. The differences were most pronounced for a_1, and least for a This discrepancy increased as the external measurements were more peripherally performed.

Discussion

Peripheral as well as central blood radioactivity was estimated by sampling blood from the tail and thoracic cavity It was noticed that the radioactivity curve in the capillary bed differed slightly from that of the big central vessels. This phenomenon was due to the fact that the substance was removed from, *and* diluted in, the blood.

The uptake of Radio-Hippuran into the red blood cells *in vivo* agreed on the whole with the *in vitro* experiments. Slightly more radioactivity was recovered in the erythrocytes *in vivo* than *in vitro* this can be explained by the lag time of the backflow of radioactivity from the red blood cells to the plasma *in vivo*. The results agreed largely with those found *in vivo* in dog and human by Smith *et al.* (1945) Since the content of radioactivity in the red blood cells changed at a rate similar to that of the whole blood, no

4 Electrophoresis. In the three buffer solutions, fraction A and fraction B wandered as free substances faster than the serum proteins. This fact was established in sera from rats injected with the tracer dose as well as the carrier dose of Radio-Hippuran.

External scintillation measurements over the heart, head, and tail

External measurements over regions representing different circulatory conditions, were performed during 110—300 minutes after injection of Radio-Hippuran, fraction A or fraction B. Six animals were investigated after injection of Radio-Hippuran. Two rats were injected with fraction A, and two with fraction B and measurements carried out only over the heart.

At the end of all experiments, RISA was injected with the detectors in the same positions as during the previous checking, and the measurements continued for about another 10 minutes.

Results

The ratio of Radio-Hippuran to RISA from the external measurements over the heart is presented in fig 15 B and from the radioactivity estimations over the head and the tail in fig 15 C and are expressed in per cent of the administered dose. In fig 15 B the external measurements are also shown when fractions A and B were used.

Heart area curves. Immediately after the injection of each of the three compounds, a spike amounting to 150—200 per cent was observed, followed by a drop to different levels, 40—50 per cent, when Radio-Hippuran and fraction A were injected, and about 60 per cent when fraction B was administered. Thereafter during 60—100 minutes, a falling phase characterized by a continuously diminishing rate was found. From 60—100 to 300 minutes the radioactivity disappeared exponentially Compared with the radioactivity curves obtained by blood sampling, the external tracing occurred at a 4—6 times higher percentage level. The relations between the external scintillation curves, obtained after injection of Radio-Hippuran, and fractions A and B were similar to those between the blood sample curves after injection of the same compounds.

External measurements over the head. After an initial rise up to 70—110 per cent, the curves dropped at an ever diminishing rate during the first 50 minutes. After this time the decrease occurred exponentially The percentage level of the last slope of the curves was 10—12 times higher than that of the blood sample curves.

External measurements over the tail. These curves began with an uptake phase during 4—10 minutes. After a maximal value of 40—65 per cent a diminishing phase followed, which could be resolved in two components with various disappearance rates, the lowest of which agreed fairly well with the corresponding phase of the blood sample curve. The tail curves were, however situated at a percentage level 20—30 times higher than the blood sample curves.

Interpretation of the radioactivity curves

It was desirable to find out whether or not the different radioactivity curves were composed of different exponential functions according to the theory of different compartment distribution or other expo-

firm binding of Radio-Hippuran in the red blood cells can have existed.

The examination of the binding of Radio-Hippuran to the serum proteins *in vitro* revealed that, like the experiments *in vivo*, either none at all or only very small amounts of Radio-Hippuran combined with the serum proteins.

It has usually been assumed that external measurements over appropriate parts of the body reflect the changes of radioactivity in the circulating blood. Since the slopes of the radioactivity in blood and in external measurement curves agree reasonably well, this has been considered evidence in support of this assumption. In the present investigation the k values from the curves recorded over the heart and head are of the same magnitude as those of the blood sample curve. This might serve as an indication that the above mentioned hypothesis is valid.

By using a reference substance in external measurements, the author has obtained quantitative values of the parameters. The fact that the values of the parameters a, a_1, and of the external measurement curves exceeded those of the corresponding parameters of the blood sample curves after injection of Radio-Hippuran, indicates that the biodynamics of the radioactivity did not occur only between the blood and the kidneys. Either it may be specific uptake of Radio-Hippuran in some tissues or a diffusion of the labelled compound in the extracellular space from which the radioactivity disappears at a rate similar to that of blood. Since no specific uptake in organs in the regions exposed to the scintillation detector was found in the distribution studies, and as Radio-Hippuran was found to exist unbound in the serum, diffusion into the extracellular space seems to be the most probable explanation. The ratio of the parameter in the external measurements to the corresponding parameter in the blood sample curve differs over various examined regions of the body. This observation can be explained by different relative amounts of extracellular radioactivity and blood radioactivity exposed to the scintillation detector. The uptake phase of the curve obtained by external measurements over the tail may be a result of very slow migration of Radio-Hippuran into the extracellular space. In contrast, the curve over the head indicates a rapid diffusion into the extracellular space.

Summary

1. The red blood cells took up 25—40 per cent of Radio-Hippuran *in vitro* and nearly as much was found to enter into the red cells in the *in vitro* experiments. The disappearance curves of Radio-Hippuran in blood and serum *in vivo* had identical slopes. This indicated that the outflow of Radio-Hippuran from the red blood cells was almost entirely unhindered.

2. Radio-Hippuran either failed completely to combine with the serum proteins, or did so only to a small extent.

3. The blood disappearance curves of injected Radio-Hippuran, fraction A, and fraction B, obtained from tail blood, agreed fairly well with the thoracic blood sample curve.

4. Fraction A and fraction B showed a lower and higher radioactivity retention respectively in blood as compared with that of Radio-Hippuran.

5. The disappearance curves of Radio-Hippuran and fraction A, and of fraction B could be expressed as a tri-ex-

Table VI Characteristics of radioactivity curves after intravenous administration of Radio-Hippuran, fraction A, and fraction B in normal rats. Phases 1 2 and 3 represent the fastest, average, and slowest exponential disappearence phases, respectively In the curve obtained from the external measurements over the tail the phase 1 represents an uptake instead of a disappearance phase. The k values denote fractions of the residual radioactivity disappearing (in the external measurements over the tail, radioactivity taken up) per minute. The a-values show the percentage values of the phases extrapolated to zero time. In the blood sample and external measurement curves the dose administered and the radioactivity level of RISA, respectively are set at 100 per cent. Values after ± are standard deviations

	Administered solution	Phase 1		Phase 2		Phase 3	
		k_1	a	k	a_2	k_3	a
Tail blood sample curves	Radio-Hippuran	0.267±0 062	16 4±6 1	0 0248±0 0055	7 1±2.1	0 00114±0 00030	1.3±0.2
Thoracic blood sample curve	Radio-Hippuran	0 190	15 0	0 0304	7 4	0 00140	1 0
Tail blood sample curve	Fraction A	0 173 0 173	12.0 11 0	0 0187 0 0204	3.2 3 0	0 00036 0 00085	0.25 0 28
Tail blood sample curve	Fraction B			0 0139 0 0306	9 0 11 3	0 00012 0 00050	7.2 7 8
External measurements over the heart	Radio-Hippuran	0 347±0 139	31±8	0 0297±0 0060	23±3	0.00122±0 00060	7 0±1 3
External measurements over the head	Radio-Hippuran	0.301±0 236	56±25	0 0346±0 0124	51±21	0 00129±0 00052	15.5±3 4
External measurements over the tail	Radio-Hippuran	0 173±0 056	36±20	0 0541±0 0233	42±20	0 00095±0 00018	34±10

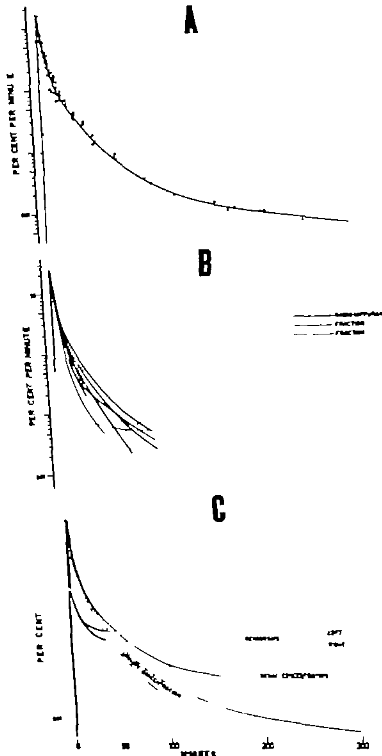


Fig. 17 Urinary concentration curves estimated from urine sampling (A) and from the external measurement over the urinary bladder (B) after intravenous injection of Radio-Hippuran, fraction A and fraction B into normal rats. I C, the mean excretion rate curves in A and B are plotted in the same diagram as the radiorenogram curves and the real renal concentration curves in A and C refer to the conditions after injection of Radio-Hippuran.

ponential and bi-exponential function, respectively

6. The curves from the external measurements over the heart and head after injection of Radio-Hippuran had a shape almost similar to that of the blood sample curve. The external measurements over the tail showed an initial uptake phase followed by a falling slope.

7. The external measurements over the heart, head and tail could be expressed as exponential functions. The external measurements over the heart and head consisted of three disappearance phases and the external measurements over the tail of one build up and two disappearance phases

8. The constant of the disappearance phase of the external measurement curves was in good agreement with that of the corresponding phase of the blood sample curves. The parameters of the disappearance phases of the external measurement curves consistently showed considerably higher values than the corresponding parameters of the blood sample curves. This fact was probably due to escaping of the Radio-Hippuran into the extracellular space.

Part 3

Excretion of Radio-Hippuran through the kidneys

The excreted amounts and the elimination rate of radioactivity into urine were examined by urine sampling and external measurements over the urinary bladder and over the kidney areas. Radiochemical analyses of the excreted radioactivity were performed. The radioactivity changes in the kidneys were registered by external radioactivity tracings over the renal areas.

Urine sample curves

Urine samples were collected during 300, 250 and 30 minutes in four, two, and one rats, respectively, after injection of Radio-Hippuran.

Results

The excreted radioactivity amounted to 20.6 ± 9.7 per cent at 4 minutes, 53.7 ± 6.1 per cent at 10 minutes, 77.3 ± 6.2 per cent at 30 minutes, 88.3 ± 2.9 per cent at 100 minutes, and 92.0 ± 2.1 per cent of the injected dose at 300 minutes after injection of Radio-Hippuran.

The urinary elimination of radioactivity is shown in fig. 17 A as per cent of administered radioactivity per minute. A maximal excretion rate amounting to 11.2—16.0 per cent per minute was found at 2.5—4.5 minutes. Thereafter there was a continuous decrease in the urinary radioactivity content, with values of 0.01—0.03 per cent per minute at 300 minutes.

Radiocystogram curves

External measurement over the urinary bladder was performed in rats after injection of the following solutions

Radio-Hippuran. External scintillation measurement over the urinary bladder was carried out in eight normal rats, two of which were killed at 30 and one at 100 minutes. The radioactivity excreted in the urine of these three rats was determined

graphy beta scanning, and direct measurement of pieces of paper strips or sheets.

Cross-matching experiments were carried out in the two chromatographic systems and in electrophoresis with buffer solution I and by using the following urine samples and mixtures

I. Urine samples taken at 240 minutes from two rats injected with a tracer dose of Radio-Hippuran.

II. Urine from an untreated rat, mixed with Radio-Hippuran in amounts giving the same concentration of Radio-Hippuran as in urine I.

III. Urine from an untreated rat, mixed with $^{131}I^-$ giving the same concentration as fraction B of urine I.

IV. Urine I mixed with an equal volume of a solution containing Radio-Hippuran in the same concentration as component A of *urine* I.

V. Urine I mixed with an equal volume of a solution containing $^{131}I^-$ in the same concentration as fraction B in urine I.

VI. Urine removed at 120 minutes after intravenous injection of a tracer dose of $Na^{131}I$.

Urines I, II, III and VI were applied to the paper in amounts of 0.01 ml and urines IV and V in amounts of 0.02 ml.

Urines I and V were also examined in two-dimensional chromatography as described in Chapter IV.

Results

Two fractions appeared in urine, the *Rf*-values and the velocities of which in the chromatographic systems and in electrophoresis respectively corresponded to fractions A and B in the stock solutions of Radio-Hippuran. At the place corresponding to the site of fraction C no radioactivity appeared in urine. The small fractions which appeared upon analyses of Radio-Hippuran in chromatography with solvent II were recovered in urine.

In cross-matching experiments (fig. 18) Radio-Hippuran, or Radio-Hippuran added to urine from tested rats, did not

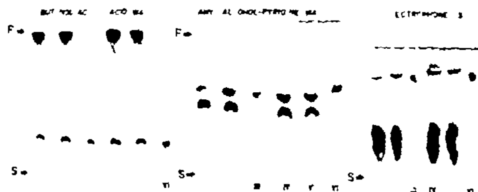


Fig. 18. Autoradiograms of uni-dimensional paper chromatography in two solvents and electrophoresis of Radio-Hippuran (I) and of $Na^{131}I$ (VI) excreted in urine. Autoradiograms (II) and (III) respectively refer to Radio-Hippuran and $Na^{131}I$ mixed with urine from an untreated rat. Autoradiograms (IV) and (V) refer respectively to cross-matching of (I) and Radio-Hippuran, and of (I) and $Na^{131}I$. S = starting point. F = solvent front.

by direct measurement of the removed urinary bladder In five other animals the total excreted radioactivity was calculated from a urine sample taken at about 100—150 minutes (see Chapter VI) When performing the radiocystogram on the rat killed at 100 minutes, another apparatus for external measurement, connected with the gamma ray spectrometer measured from below the radioactivity in the urinary bladder

Fraction A Four rats, killed at 30 minutes after injection.

Fraction B Two rats, killed at 300 minutes after injection.

Urine from a rat injected with Radio-Hippuran The radiocystogram curves were performed in two rats during 75—90 minutes after injection of the radioactive urine. The excreted radioactivity was determined from a urine sample taken at the end of the experiment.

Results

Radio-Hippuran The amount of radioactivity excreted in the urine of five rats by 100 minutes, calculated from a urine sample, was 90 2 ± 1 6 per cent. The radioactivity in the urinary bladder estimated from the radiocystogram curve as described in Chapter VI amounted to 41 8 ± 9 3 at 4 minutes, 70 4 ± 5 6 at 10 minutes, and 82 7 ± 5.2 per cent of given dose at 30 minutes. These values did not differ significantly from those found in the distribution studies.

The shape of the cystogram curve obtained in connection with the gamma ray spectrometer corresponded to that of the curve obtained without the spectrometer

The excretion rate of radioactivity is presented in fig 17 B. The form of this curve was similar to that of the urine sample curve. The highest output rate occurred at 1.5—4 minutes and amounted to 13.5—23 8 per cent per minute. Thus the excretion velocity was somewhat higher than in the urine sample curve.

Fraction A The concentration curves are given in fig 17 B and they corresponded in shape to those of Radio-Hippuran. The maximum, 15.3—20 0 per cent per minute, occurred at about 2.5 minutes.

Fraction B The results are shown in fig 17 B

After an initial maximum (0.2—0.5 per cent per minute) of the excretion curve at the same time interval as that when Radio-Hippuran and fraction A were used, the rate of urinary elimination became rather constant. A second maximum of about 0 1 per cent per minute was suggested at 110—165 minutes.

Radioactive urine In the two rats, the radioactivity excreted into the urinary bladder according to calculation from the radiocystogram curve, amounted to 39.2 and 30.2 per cent at 4 minutes, 75 0 and 62 6 per cent at 10 minutes, 90.7 and 85 6 per cent at 30 minutes, and 90.8 and 91.3 per cent at 75—90 minutes. A maximal excretion rate of about 11 6 per cent per minute occurred at 2.5—3.5 minutes.

Chromatographic and electrophoretic analyses of radioactivity excreted in urine

Urine samples collected at various time intervals from 30 to 300 minutes after injection of Radio-Hippuran into 15 rats were examined with one-dimensional chromatographic and electrophoretic technique described in Chapter IV The radioactivity was localized with autoradio

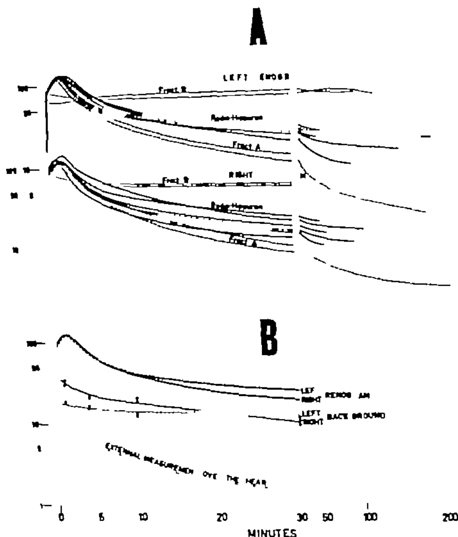


Fig 19 Radiorenogram curves (A) from normal rats after injection of Radio-Hippuran, fraction A and fraction B. Left and right background curves over areas of removed kidneys in relation to radiorenogram curves and external measurements over the heart (B) after administration of Radio-Hippuran to normal animals. The outer numbers on the ordinate axis refer to the right radiorenogram curves.

Influence of the radioactivity of surrounding tissues on the renogram curves

In comparing the renogram curves with the real renal concentration course determined by direct measurement (fig. 17 C) an evident divergence was detected after 2—3 minutes. This may be due to the fact that the changes in the radioactivity in surrounding tissues and organs influenced the renogram curve. An attempt to determine this background curve was made.

cause any appearance of new fractions, but, instead, accumulations of the added radioactivity to that of the original fractions of the urine from injected animals. Thus it is very probable that fractions A and B of Radio-Hippuran were excreted unchanged into the urine.

Quantitative measurements of the radioactivity in the two components of the urine revealed that urine contained smaller amounts of fraction B than did the administered solution of Radio-Hippuran, which may indicate a slower excretion rate of this component than of fraction A. By following up the amounts of excreted fraction B it was shown that at 30 150 and 240—300 minutes, 0—20 23—53 and 27—58 per cent, respectively of fraction B in Radio-Hippuran, was eliminated into the urine. Thus, the amounts of excreted fraction B determined in this way were somewhat larger than those obtained in the distribution studies (table V)

Radiorenogram curves

External measurements over each kidney area under standard conditions were performed after injection into rats of the following solutions

Radio-Hippuran. Renograms were performed over six left and six right kidneys during 30—270 minutes. When an ordinary left-side renogram was carried out from below another radiorenogram was simultaneously taken from the same kidney from above with a gamma ray spectrometer

Fraction A Renograms were taken from two left and two right kidneys during 30—210 minutes.

Fraction B. Renograms from two left and two right kidneys were measured during 30—120 minutes.

Urine from a rat injected with Radio-Hippuran. Left-side renograms were performed in two rats examined during 100—180 minutes.

Results

The scintillation curves obtained after injection of Radio-Hippuran and fractions A and B correlated to the same amount of radioactivity administered, are presented in fig 19 A.

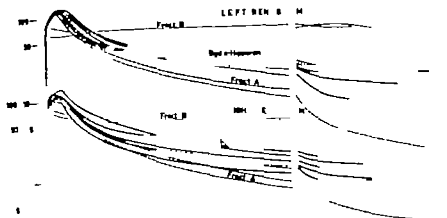
Radio-Hippuran. On comparison of the renograms from each side, the right radiorenogram curves showed a tendency to accumulate more radioactivity in the uptake phase and a faster decreasing rate of the excretion phase, resulting in a significantly lower radioactivity level on this side at 30 minutes ($P < 0.05$) The shapes of the two renograms performed simultaneously with and without gamma ray spectrometry were in good accordance with each other

Fraction A. The renogram curves from both sides agreed well with each other Owing to a pronounced diminishing rate of the excretion segment, they deviated at 7—21 minutes from the Radio-Hippuran renogram curves, especially on the left side.

Fraction B. These curves were characterized by slowly occurring changes. After a downward slope during the first seven minutes the curves remained at a rather constant level on the right side, while, after about 1—2 minutes, the left side renograms showed an accumulation phase lasting between 30 and 75 minutes.

Radioactive urine The renogram curves were similar to those obtained after injection of Radio-Hippuran and fraction A in respect to both shape and amplitude.

A



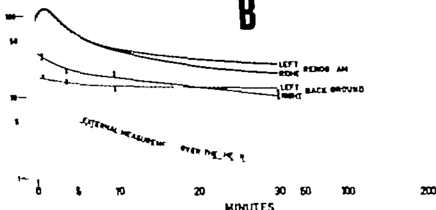


Fig 19 Radiorenogram curves (A) from normal rats after injection of Radio-Hippuran, fraction A and fraction B. Left and right background curves over areas of removed kidneys in relation to radiorenogram curves and external measurements over the heart (B) after administration of Radio-Hippuran to normal animals. The outer numbers on the ordinate axis refer to the right radiorenogram curves.

Influence of the radioactivity of surrounding tissues on the renogram curves

In comparing the renogram curves with the real renal concentration course determined by direct measurement (fig 17 C) an evident divergence was detected after 2—3 minutes. This may be due to the fact that the changes in the radioactivity in surrounding tissues and organs influenced the renogram curve. An attempt to determine this background curve was made.

cause any appearance of new fractions, but, instead, accumulations of the added radioactivity to that of the original fractions of the urine from injected animals. Thus it is very probable that fractions A and B of Radio-Hippuran were excreted unchanged into the urine.

Quantitative measurements of the radioactivity in the two components of the urine revealed that urine contained smaller amounts of fraction B than did the administered solution of Radio-Hippuran, which may indicate a slower excretion rate of this component than of fraction A. By following up the amounts of excreted fraction B it was shown that at 30 150 and 240—300 minutes, 0—20 23—53 and 27—58 per cent, respectively of fraction B in Radio-Hippuran, was eliminated into the urine. Thus, the amounts of excreted fraction B determined in this way were somewhat larger than those obtained in the distribution studies (table V)

Radiorenogram curves

External measurements over each kidney area under standard conditions were performed after injection into rats of the following solutions

Radio-Hippuran. Renograms were performed over six left and six right kidneys during 30—270 minutes. When an ordinary left-side renogram was carried out from below another radiorenogram was simultaneously taken from the same kidney from above with a gamma ray spectrometer

Fraction A Renograms were taken from two left and two right kidneys during 30—210 minutes.

Fraction B. Renograms from two left and two right kidneys were measured during 30—120 minutes.

Urine from a rat injected with Radio-Hippuran. Left-side renograms were performed in two rats examined during 100—180 minutes.

Results

The scintillation curves obtained after injection of Radio-Hippuran and fractions A and B correlated to the same amount of radioactivity administered, are presented in fig 19 A.

Radio-Hippuran. On comparison of the renograms from each side, the right radiorenogram curves showed a tendency to accumulate more radioactivity in the uptake phase and a faster decreasing rate of the excretion phase, resulting in a significantly lower radioactivity level on this side at 30 minutes ($P < 0\,05$) The shapes of the two renograms performed simultaneously with and without gamma ray spectrometry were in good accordance with each other

Fraction A The renogram curves from both sides agreed well with each other Owing to a pronounced diminishing rate of the excretion segment, they deviated at 7—21 minutes from the Radio-Hippuran renogram curves, especially on the left side.

Fraction B. These curves were characterized by slowly occurring changes. After a downward slope during the first seven minutes the curves remained at a rather constant level on the right side, while, after about 1—2 minutes, the left side renograms showed an accumulation phase lasting between 30 and 75 minutes.

Radioactive urine The renogram curves were similar to those obtained after injection of Radio-Hippuran and fraction A in respect to both shape and amplitude.

revealed that Radio-Hippuran was very probably excreted unchanged. This is in accordance with the suggestions of Swick (1933) who considered that conjugation with glucuronic acid was unlikely. It was also shown that fraction B, $^{131}I^{-}$ was excreted unaltered. Any acceptable explanation as to why this fraction was eliminated in larger amounts than could be calculated from the distribution studies (table V) is difficult to give at present. Decomposition of fraction A in vivo might be an explanation.

The disagreement between the radiorenogram curve and the real changes of the renal concentration of Radio-Hippuran was shown to depend on the influence of *the radioactivity of the surrounding tissues and organs*, on the right side, especially from the liver during the first 10 minutes, and on the left side, after about 10 minutes, from the contents of the stomach and the small intestine. The somewhat higher level of the uptake phase of the right renogram, and the higher level of excretion phase of the left renogram at 30 minutes, were revealed to depend on the influence of the background.

By performing radiorenogram curves with use of separated fractions A and B, it was established that fraction B caused disturbance of the excretion phase of the renogram by diminishing the disappearance rate of the excretion phase especially on the left side. This was due to the excretion of component B in the stomach and in the small intestine.

Since the background curves from each side differed and did not correspond to the blood sample curv concerning the disappearance rates, the calculation of the renal concentration curve by subtracting the blood background from the original renogram is not quite exact.

A deviation between the real renal and the urine ^{131}I concentration curves exists. After 30 minutes the concentration in kidneys diminished more slowly than in the urine. The cause may be an accumulation of ^{131}I in the kidneys.

By the external measurements over the kidney and the urinary bladder performed with gamma ray spectrometry it was established that the radioactivity courses cannot be explained by an effect of scattered radiation.

Summary

1. The amount of ^{131}I excreted into urine after injection of Radio-Hippuran determined from the radiocystogram curv agreed very well with the radioactivity value obtained in the distribution studies. The urinary radioactivity estimated by urine sampling differed to some degree from the results in the distribution studies.

2. After the injection of Radio-Hippuran the urinary excretion rate curve showed a maximal excretion rate at 1.5—4.5 minutes. The urinary excretion rate curve of fraction A corresponded well to that obtained after injection of Radio-Hippuran. The elimination of radioactivity into the urine after injection of fraction B occurred t slow rate and at a rather constant level.

3. The Radio-Hippuran *renogram curve* from the right side showed a tendency to a higher level during the uptake phase and a significantly lower value during excretion, according to measurements at 30 minutes after injection of Radio-Hippuran.

4. The background radioactivity after injection of Radio-Hippuran, measured over the renal areas after removal of the

Two rats were killed by strangulation of the vessels of the heart root at each of the following time intervals after injection of Radio-Hippuran 1 4 10 and 30 minutes. After removal of the kidneys the radioactivity was measured over the renal areas as described in Chapter VI. The measurement was repeated after removal of other organs (liver stomach, and small intestine)

Results

The radioactivity levels of the "background" at the examined time intervals are shown in fig 19 B

The right background curve was reminiscent of the course of the radioactivity changes obtained from the blood sample curves as well as the external measurements over the heart and head, but differed from these curves by a slower diminishing rate. The left curve started at a lower level than the right and showed a tendency to increase after 10 minutes.

Comparison of the urine concentration curves and the radioactivity content in the kidneys

In order to examine whether or not the urinary excretion rate curve reflects the radioactivity content in the kidneys, the mean values of the radioactivity in one kidney at the six time intervals in the distribution studies, and mean values of urinary excretion rate curves obtained by urinary sampling as well as calculated from the radiocystograms, are plotted in a semilogarithmic system as shown in fig 17 C. Upon comparison of the decreasing rates of the curves it was noticed that they corresponded to each other rather well during the first 30 minutes, but after this time the renal concentration curve deviated due to slower disappearance of radioactivity from the kidneys.

Discussion

It is desirable in many clinical and scientific investigations to continuously measure the excretion into urine of an administered compound. Hitherto, the only suitable method has been to perform continuous urine sampling by bladder catheterization, which is accompanied by some errors and risks. By introduction into the body of a compound labelled with a gamma radiating isotope, the time-concentration curve of the radioactivity in the urinary bladder can be obtained by external measurement over the urinary bladder in a way similar to that of radiorenography Correlation of these results, as described in Chapter VI yielded a rather good agreement with those obtained in the distribution studies. The low values of excreted amounts of Radio-Hippuran, according to the estimations of urine samples, were probably due to disturbance of the excretion by manipulation of the bladder The initially high excretion velocity of Radio-Hippuran in urine when fraction B was injected seems to be due to the excretion of small amounts of fraction A, which amounted to 1 9—2 4 per cent of the radioactivity in the injected solution.

Urine from one rat injected with tagged ortho-iodohipppurate, was injected into another rat, the excretion rate into urine followed by radiocystogram curve, and the accumulation of radioactive content in the kidney by radiorenogram. The results of this investigation and those of radiochemical analyses with cross-matching experiments of radioactivity excreted into urine after injection of Radio-Hippuran have

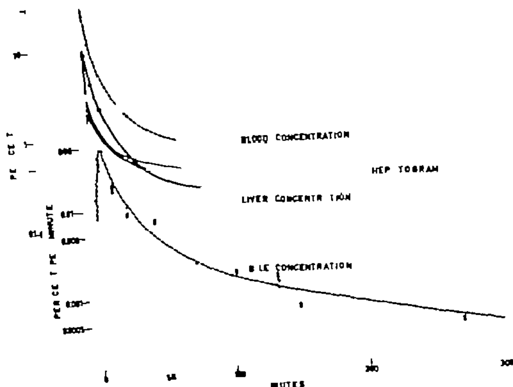


Fig 20 Time-intensity curves of radioactivity in bile, liver and blood, and hepatogram curves after intravenous injection of Radio-Hippuran in normal rats. Right ordinate axis refers to bile radioactivity concentration, and left ordinate axis to blood and liver radioactivity concentration. The hepatogram curves are expressed in relative counts per time unit and plotted in the same semilogarithmic system as the other curves.

minishing of the excretion rate agreed during the first 100 minutes w th that of the liver and blood concentration, and thereafter occurred somewhat faster than those.

The radioactivity of the small intestine, wall and contents, amounted to 0 34 ± 0 03 and 0 60 ± 0 44 per cent of given dose respectively The total gastrointestinal tract, excluding the bile contained 3.37 ± 0 48 per cent of the injected radioactivity These values did not significantly differ from those found in the distribution tudies Nor did the radioactivity of the liver t 300 minutes differ significantly from the values found in non-catheterized rats.

Radiohepatogram curves

In three rats, external measurements were performed over the liver area during 200—220 minutes after the administration of Radio-Hippuran.

Result

The course of radioactivity changes over the liver was similar to that obtained from the external measurements over the head.

kidney was found to have a slower disappearance rate than the blood sample curves. The influence of the liver radioactivity caused a higher radioactivity level on the right side during the first ten minutes. After 30 minutes the left side background radioactivity was higher than the right one. The differenses of the background curves seem to explain the divergence of the renogram curves from both sides.

5. The radiorenogram curves performed after injection of fraction A were identical to that of Radio-Hippuran during the uptake phase but differed later by a higher decreasing rate of the excretion phase, probably because of the influence of smaller amounts of radioactivity in the gastrointestinal tract. The radioactivity courses over the kidneys, after administration of fraction B were characterized by a rather constant level. On the right side they had a slowly diminishing slope, and on the left side, an increasing tendency mainly reflecting the amounts of ^{131}I in the gastrointestinal tract. The behaviour of fraction B in this respect explains the differences in the levels of the excretion phases of the renogram curves when Radio-Hippuran and fraction A were injected.

6. The urinary concentration curve agreed with the real renal concentration curve during the first 30 minutes after which time the urinary curve decreased at a faster rate.

7. Radiochemical analyses of the radioactivity excreted into urine as well as study of biologic behaviour of the excreted ^{131}I indicated that component A was eliminated unchanged into urine. The radiochemical investigation showed also that component B was excreted as $^{131}I^-$ and at a slower rate than fraction A.

Part 4

Excretion of Radio-Hippuran through the liver and external measurements over the thyroid gland

The amounts of ^{131}I excreted into bile were determined by bile sampling. In order to check the liver concentration of ^{131}I external measurements over the liver area were performed. Radioactivity tracings over the thyroid gland were carried out to establish if there was an uptake of ^{131}I in this organ.

Bile sampling

The excretion of ^{131}I into bile was examined in six rats during 230—300 minutes after injection of Radio-Hippuran. When the bile sampling was ended, the animal was killed and the radioactivity of the liver, the small intestine, and the total gastrointestinal tract was measured as in the distribution studies.

Results

During 300 minutes a mean value of 1.09 ± 0.33 per cent of injected Radio-Hippuran was recovered in bile, the main part of which was excreted during the first 60 minutes.

In fig. 20 the bile concentration is presented. A maximal excretion rate of 0.07—0.03 per cent per minute at 5—10 minutes was shown, followed by a gradual decrease of the elimination rate. This di-

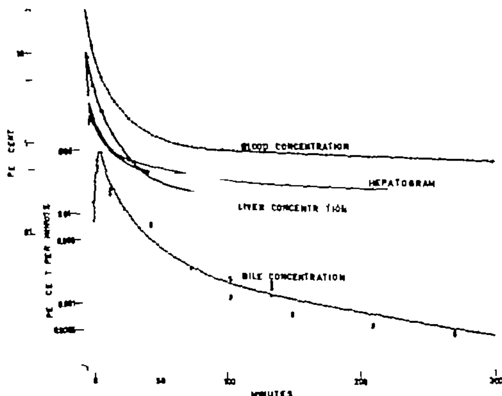


Fig 20. Time-intensity curves of radioactivity in bile, liver and blood, and hepatogram curves after intravenous injection of Radio-Hippuran in normal rats. Right ordinate axis refers to bile radioactivity concentration, and left ordinate axis to blood and liver radioactivity concentration. The hepatogram curves are expressed in relative counts per time unit and plotted in the same semilogarithmic system as the other curves.

minishing of the excretion rate agreed during the first 100 minutes with that of the liver and blood concentration, and thereafter occurred somewhat faster than those.

The radioactivity of the small intestine, wall and contents, amounted to 0.34 ± 0.05 and 0.60 ± 0.44 per cent of given dose respectively. The total gastrointestinal tract, excluding the bile contained 3.37 ± 0.48 per cent of the injected radioactivity. These values did not significantly differ from those found in the distribution studies. Nor did the radioactivity of the liver at 300 minutes differ significantly from the values found in non-catheterized rats.

Radiohepatogram curves

In three rats, external measurements were performed over the liver area during 200—220 minutes after the administration of Radio-Hippuran.

Results

The course of radioactivity changes over the liver was similar to that obtained from the external measurements over the head.

Absorption in the small intestine of radioactivity excreted into bile

The sum of the radioactivity recovered in the contents of the small intestine and in the bile, in rats with a catheter in the common bile duct, exceeded the radioactivity in the contents of the small intestine in non-catheterized rats. Thus, an absorption of the bile radioactivity in the small intestine may be suggested. To verify this hypothesis, the following experiments were performed. Bile collected during 100 minutes after injection of Radio-Hippuran in a bilaterally nephrectomized rat was injected into the small intestine of two normal rats. The animals were killed after 300 minutes and the radioactivity of the removed urinary bladder and different parts of the gastrointestinal tract measured.

Results

The radioactivity remaining in the small intestine amounted to 37.2 and 37.0 per cent. In the urinary bladder 57.9 and 52.0 per cent of the given dose was recovered in the two rats.

External measurements of the thyroid gland

Two normal rats were examined during 200—210 minutes after injection of Radio-Hippuran.

Results

After a falling phase during 40—75 minutes a slow uptake phase followed during the rest of examination time.

Discussion

The amounts of ^{131}I excreted into bile exceeded somewhat those found by zum Winkel and de Maria (1961). They found about 0.6 per cent in a duodenal pouch at 120 minutes after injection of Radio-Hippuran into rats, while in the present investigation an average of 1.06 per cent was excreted during the same interval.

Since the disappearance rates of the curves from the radiohepatograms, the liver and the bile radioactivity concentration correspond, on the whole, to that of the blood radioactivity concentration, it may be suggested that no active uptake or excretion, but a passive diffusion or filtration exists in the liver. In comparing the real concentration of the radioactivity in the bile and the whole blood, a concentration gradient, bile to blood, of 3.1, 3.1 and 1.6 at 10, 100 and 300 minutes, respectively indicates that an active uptake and excretion of Radio-Hippuran occur in the liver.

The results of the external measurements over the thyroid gland supported the hypothesis derived from the results, obtained by direct measurements, that an accumulation of ^{131}I occurred in this organ.

Summary

1. Bile measurements in normal rats showed that small amounts of ^{131}I, about 1 per cent during 300 minutes, were excreted through the liver after injection of Radio-Hippuran. The bile concentration and liver concentration as well as radiohepatogram curves had a similar diminishing rate to that of blood. A concentration gradient, bile to blood, of 3.1—1.6 was detected, indicating an active liver uptake and excretion of Radio-Hippuran. An absorption of the radioactivity in bile probably occurred in the gastrointestinal tract.

2. External measurements over the thyroid gland verified that an uptake of ^{131}I exists after injection of Radio-Hippuran.

CHAPTER VIII

Behaviour of Radio-Hippuran in unilaterally nephrectomized rats

Part 1

General distribution

One or two days after unilateral nephrectomy rats were subjected to distribution studies after injection of Radio-Hippuran. Tw left and two right-side nephrectomized rats were killed at each of the following time intervals 1 4 10, 30, 100, and 300 minutes. The radioactivity of organs assumed to accumulate, excrete, or metabolize Radio-Hippuran was measured in all animals, while that of other organs was checked only in two animals t each time interval.

Results

Data from the scintillation measurements are show in table VII

The results indicated principally distribution of Radio-Hippuran in the unilaterally nephrectomized rats similar to that in the normal condition. Most of the radioactivity was recovered in the rinary bladder after 30 minutes. A small portion was excreted into the gastrointestinal tract, and recovered mainly in the contents of the stomach and the small intestine An uptake was noticed in the thyroid gland. The radioactivity in the kidneys decreased faster than in blood, but in most of the other organs at nearly the same rate as in blood.

The results from rats examined one and two days after nephrectomy did not differ from each other

Discussion

It has long been known that kidney-specific substances, administered to the body or endogenously produced, are retained in blood when the kidney function is impaired. To what extent the loss of the function of one kidney may be reflected in the retention of kidney-specific substances, has been examined by several a thors (Braun-Menéndez and Chiodi, 1947 W tchinger and Werner 1949 Weiss and Chaus, 1945 Malm, 1949 and others) I these studies, mainly clearance technique has been used. Rollason (1949) concluded that hypertrophy following uninephrectomy begins in the rat at one—two days after the operation with enlargement of the tubular cells and increased frequency of mitoses. Braun-Menéndez and Chiodi (1947) reported that uninephrectomy in

Table VII Content of radioactivity in various organs of unilaterally nephrectomized rats, 1 4 10 30, 100 and 300 *minutes after intravenous injection of Radio-Hippuran.* The values show the percentage of the dose administered and the standard deviations

Organ	1 minute	4 minutes	10 minutes	30 minutes	100 minutes	300 minutes
Remaining kidney	*18.32±2 64*	*8.22±2 19*	*4 13±1 42*	*1.30±0.35*	0.34±0.24	0.10±0.04
Urinary bladder	4 74±5 17	32.22±3.87	58.23±7.89	75 19±7.92	82.89±4.52	88.88±1.60
Blood	30.90+3.38	17 18±1.93	8.94±1.27	4 03±0 35	1.52±0.25	0.80±0.37
Thyroid gland	0.25±0 09	0 17±0.04	0.19±0 03	0.33±0.26	0.52±0 13	1.30±1.39
[illegible]	[illegible]	5.90±0.78	3.11±0.86	1.34±0.28	0 43±0 04	0.23±0.05
[illegible]		0 68±0 15	0.57±0 08	0.44±0.24	0.26±0.14	0.23±0.12
[illegible]		0.49±0.23	0.78±0.31	0.98±0 41	1 16±0.53	0.94±0.32
[illegible]		2 63±0.36	1 60±0.54	1.64±0.29	0.81±0.22	0 70±0.35
» » contents	1.21±0 45	1.29±0.58	1 05±0.53	1.69±0.36	1 47±0.44	1.58±0.66
Coecum wall	0 63±0.28	0 44±0 09	0.26±0 05	0.14±0 03	0 05±0.03	0.06±0.02
» contents	0.24±0 13	0 15±0 05	0.09±0 04	0 11±0 05	0 13±0 11	0.19±0 16
Colon wall	0.91±0.22	0 60±0 12	0.34±0 06	0.22±0.04	0 10±0 07	0.05±0.01
» contents	0 08±0 02	0 14±0 17	0.07±0 07	0 07±0.02	0.22±0.35	0 09±0.09
Total gastrointestinal tract	7 48±1 47	6.41±1 16	4 76±1.27	5.29±0 48	4 19±1 47	3.84±1.25
Spleen	0 40 0.33	0.20 0 15	0 15 0 18	0.08 0.07	0 03 0.02	0.02 0.02
Gl. parotis	0.46 0 34	0.25 0.21	0 14 0.20	0 06 0.06	0.02 0.03	0.04 0.02
Adrenal glands	0 060 0 077	0.043 0 039	0 023 0.029	0 011 0 008	0 004 0 005	0.004 0.005
Lungs	1 43 1 41	0 89 0 89	0.50 0.78	0.24 0.23	0.08 0.09	0.08 0 07
Uterus	*0 436* 0.639	*0 471* 0 669	0.227 0 413	0 094 0.104	0 115 0.085	0 083 0.053
Ovaries	0.208 0.134	0 071 0 062	0 058 0 062	0 020 0.024	0.007 *0 011*	0 004 *0.003*
Thymus	0 165 *0 133*	0 067 *0 161*	0 068 *0 077*	0.043 0 030	0 014 0.013	0.014 0.009
Skin (per gram)	0.25 0.20	0.35 0.24	0.31 0.26	0 19 0.21	0 05 0.07	0.05 0.04
Skeletal muscle (per gram)	0.18 0 16	0 17 0 17	0 14 0 12	0.07 0.08	0 02 0.05	0.02 0.01
Pancreas (per gram)	0 70 0 79	0.50 0 45	0 42 0.25	*0 09* 0 14	*0 04* 0.05	*0.01* 0.04
Brain (per gram)	*0 087* 0 085	*0 054* 0 043	0 041 0 027	0.024 0 013	0 004 0 007	0 007 0.004

the rat reduced inulin clearance and Tm for Diodrast to 60—70 per cent of normal during the first 20 days. Watschinger and Werner (1949) found that the filtration was reduced to half its normal value, while clearance and Tm for Diodrast were restored nearly to their normal values by the fourth day

In comparing the distribution of Radio-Hippuran in normal and unilaterally nephrectomized rats, the latter animals showed significantly smaller amounts of radioactivity in the urinary bladder between 4 and 100 minutes. This difference was most pronounced at 4 minutes ($P < 0.001$) On the whole, the radio-

activity in various organs and in the contents of the different parts of the gastrointestinal tract showed somewhat higher values than those observed in the normal rats. This fact was most evident in the blood radioactivity where significantly higher values were found during the first 100 minutes (at 1 and 100 minutes, $P < 0.01$ at 10 and 30 minutes, $P < 0.001$ and at 4 minutes, $P < 0.05$)

It was also noticed that the remaining kidney accumulated nearly the same amount of radioactivity at one minute, and tendentiously ($0.05 < P < 0.1$) larger amounts at the other time intervals, than a single kidney in normal rats.

A disturbing factor in comparing the results of the distribution studies is that there exists some variation in the content of fraction B in the injected commercial Radio-Hippuran.

Summary

1 Unilaterally nephrectomized rats were injected with Radio-Hippuran one to two days after the operation.

2 The radioactivity was distributed principally in the same way as in normal rats. Significantly smaller amounts of radioactivity were, however recovered in the urinary bladder during the first 100 minutes.

3 In unilateral nephrectomy significantly more radioactivity was retained in blood during the first 100 minutes than in the normal condition.

4 The amount of ^{131}I in the contents of the small intestine tended to increase in uninephrectomy as compared with that in normal rats.

Part 2

Disappearance of Radio-Hippuran from blood

Blood sample curves

Blood sample measurements were carried out during 300 minutes after injection of Radio-Hippuran into seven rats, uninephrectomized one and two days earlier

Results

The radioactivity remaining in the total blood volume obtained by blood sampling from the tail or by thoracic blood sampling, is shown in fig 21 A.

From the 100 per cent, assumed to exist in blood immediately after the injection, a very rapid decrease to 70—40 per cent was noticed in the blood sample curves. There-after followed phase characterized by a continuous diminishing of the disappearance rate for about 100 minutes, after which time an approximate exponential decrease of the blood radioactivity was observed. The course of the disappearance of radioactivity from blood obtained from the distribution studies had a shape similar to that of the tail blood sample curve, but started after the initial spike t a higher percentage level, and decreased faster so that after 4 minutes it showed a radioactivity content somewhat lower than that of the blood sample curve. From about 150 minutes the two curves decreased at nearly the same exponential rate.

Table VII Content of radioactivity in various organs of unilaterally nephrectomized rats, 1, 4, 10, 30, 100, and 300 minutes after intravenous injection of Radio-Hippuran. The values show the percentage of the dose administered and the standard deviations

Organ	1 minute	4 minutes	10 minutes	30 minutes	100 minutes	300 minutes
Remaining kidney	18.32±2.64	8.22±2.19	4.13±1.42	1.30±0.35	0.34±0.24	0.10±0.04
Urinary bladder	4.74±5.17	32.22±3.87	58.23±7.89	75.19±7.92	82.89±4.52	88.88±1.60
Blood	30.90±3.38	17.18±1.93	*8.94±1.27*	4.03±0.35	*1.52±0.25*	*0.80±0.37*
Thyroid gland	0.25±0.09	0.17±0.04	0.19±0.03	0.33±0.26	0.52±0.13	1.30±1.39
Liver	13.34±3.94	5.90±0.78	3.11±0.86	1.34±0.28	0.43±0.04	0.23±0.05
Stomach wall	0.74±0.05	0.68±0.15	0.57±0.08	0.44±0.24	0.26±0.14	0.23±0.12
» contents	0.26±0.09	0.49±0.23	0.78±0.31	0.98±0.41	1.16±0.53	0.94±0.32
Small intestine wall	3.40±0.78	2.63±0.36	1.60±0.54	1.64±0.29	0.81±0.22	0.70±0.35
» » contents	1.21±0.45	1.29±0.58	1.05±0.53	1.69±0.36	1.47±0.44	1.58±0.66
Coecum wall	0.63±0.28	0.44±0.09	0.26±0.05	0.14±0.05	0.05±0.03	0.06±0.02
» contents	0.24±0.13	0.15±0.05	0.09±0.04	0.11±0.05	0.13±0.11	0.19±0.16
Colon wall	0.91±0.22	0.60±0.12	0.34±0.06	0.22±0.04	0.10±0.07	0.05±0.01
» contents	*0.08±0.02*	*0.14±0.17*	*0.07±0.07*	*0.07±0.02*	*0.22±0.35*	*0.09±0.09*
Total gastrointestinal tract	7.48±1.47	6.41±1.16	4.76±1.27	5.29±0.48	4.19±1.47	3.84±1.25
Spleen	0.40	0.20	0.15	0.08	0.03	0.02
	0.33	0.15	0.18	0.07	0.02	0.02
Gl. parotis	0.46	0.25	0.14	0.06	0.02	0.04
	0.34	*0.21*	*0.20*	*0.06*	*0.03*	*0.02*
Adrenal glands	0.060	0.043	0.023	0.011	0.004	0.004
	0.077	0.039	0.029	0.008	0.005	0.005
Lungs	1.43	0.89	0.50	0.24	0.08	0.08
	1.41	0.89	0.78	0.23	0.09	0.07
Uterus	0.436	0.471	0.227	0.094	0.115	0.083
	0.639	0.669	0.413	0.104	0.085	0.033
Ovaries	0.208	0.071	0.058	0.020	0.007	0.004
	0.134	*0.062*	*0.062*	*0.024*	*0.011*	0.003
Thymus	0.165	0.067	0.068	0.043	0.014	0.014
	0.133	0.161	0.077	0.030	0.013	0.009
Skin (per gram)	0.25	0.35	0.31	0.19	0.05	0.05
	0.20	0.24	0.26	0.21	0.07	0.04
Skeletal muscle (per gram)	0.18	0.17	0.14	0.07	0.02	0.02
	0.16	0.17	0.12	0.08	0.03	0.01
Pancreas (per gram)	0.70	0.50	0.42	0.09	0.04	0.01
	0.79	*0.45*	*0.25*	*0.14*	*0.05*	0.04
Brain (per gram)	0.087	0.054	0.041	0.024	0.004	0.007
	0.085	0.043	0.027	0.013	0.007	0.004

the rat reduced inulin clearance and Tm for Diodrast to 60—70 per cent of normal during the first 20 days. Watschinger and Werner (1949) found that the filtration was reduced to half its normal value, while clearance and Tm for Diodrast were restored nearly to their normal values by the fourth day

In comparing the distribution of Radio-Hippuran in normal and unilaterally nephrectomized rats, the latter animals showed *significantly* smaller amounts of radioactivity in the urinary bladder between 4 and 100 minutes. This difference was most pronounced at 4 minutes ($P < 0.001$) On the whole the radio-

activity in various organs and in the contents of the different parts of the gastro-intestinal tract showed somewhat higher values than those observed in the normal rats. This fact was most evident in the blood radioactivity where *significantly* higher values were found during the first 100 minutes (at 1 and 100 minutes, $P < 0.01$ at 10 and 30 minutes, $P < 0.001$ and at 4 minutes, $P < 0.05$)

It was also noticed that the remaining kidney accumulated nearly the same amount of radioactivity at one minute, and tendentiously ($0.05 < P < 0.1$) larger amounts at the other time intervals, than single kidney in normal rats.

A disturbing factor in comparing the results of the distribution studies is that there exists some variation in the content of fraction B in the injected commercial Radio-Hippuran.

Summary

1 Unilaterally nephrectomized rats were injected with Radio-Hippuran one to two days after the operation.

2. The radioactivity was distributed principally in the same way as in normal rats. Significantly smaller amounts of radioactivity were, however recovered in the urinary bladder during the first 100 minutes.

3 In unilateral nephrectomy significantly more radioactivity was retained in blood during the first 100 minutes than in the normal condition.

4 The amount of ^{131}I in the contents of the small intestine tended to increase in uninephrectomy as compared with that in normal rats.

Part 2

Disappearance of Radio-Hippuran from blood

Blood sample curves

Blood sample measurements were carried out during 300 minutes after injection of Radio-Hippuran into seven rats, uninephrectomized one and two days earlier

Results

The radioactivity remaining in the total blood volume obtained by blood sampling from the tail or by thoracic blood sampling, is shown in fig. 21 A.

From the 100 per cent, assumed to exist in blood immediately after the injection, a very rapid decrease to 20—40 per cent was noticed in the blood sample curves. There-after followed phase characterized by a continuous diminishing of the disappearance rate for about 100 minutes, fter which time an approximate exponential decrease of the blood radioactivity was observed. The course of the disappearance of radioactivity from blood *obtained from* the distribution studies had a shape similar to that of the tail blood *sample curve, but* started after the initial spike at a higher percentage level, and decreased faster so that after 4 minutes it showed a radioactivity content somewhat lower than that of the blood sample curve. From about 150 minutes the two curves decreased at nearly the same exponential rate.

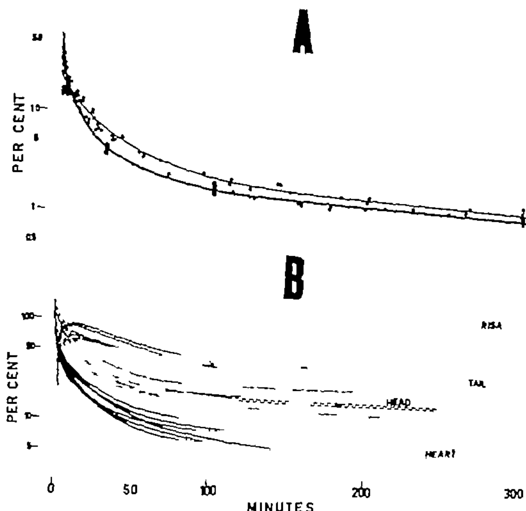


Fig 21 Time-radioactivity curves in blood (A) and over the heart, head and tail (B) after intravenous injection of Radio-Hippuran in unilaterally nephrectomized rats. In B, the radioactivity level of RISA is set at 100 per cent. In A, small dots and thin line represent tail blood samples, and big dots and heavy line thoracic blood samples.

External scintillation measurement over the heart, head and tail

Radioactivity tracings over regions representing different circulatory conditions continued for about another 10 minutes after injection of Radio-Hippuran. At the end of the Radio-Hippuran procedures, RISA was injected and the tracings were continued for about another 10 minutes with the detectors in the same positions as during the previous checking In the external measurement over the heart, head and tail, groups of six, five, and five rats, respectively were used.

Results

The external measurements over the three parts of the body are presented in fig 21 B

Measurements over the heart After an initial spike up to about 200 per cent, and decreasing to 60—70 per cent, a phase characterized by a continuous diminishing of the disappearance velocity followed,

lasting about 70 minutes. *After this time interval an approximate exponential disappearance rate of the curve occurred.*

Measurements over the head. These curves rose rapidly up to 90—115 per cent, diminished then at a continuously decreasing rate during 70 minutes, and thereafter at a rather constant exponential rate.

Measurements over the tail. During the first 5—10 minutes this course showed an uptake phase with a maximum of 60—90 per cent. After this time falling phase was seen. This decreasing segment could be divided into two parts, the first of which had a gradually diminishing falling tendency and the last of which a rather constant exponential disappearance rate.

Interpretation of the radioactivity curves

The blood sample curves as well as the external tracings were graphically analysed according to the principles described in Chapter VII.

Results

In the blood sample curves from the tail and thoracic cavity as well as in the external measurements over the heart and head, three disappearance phases 1 2, and 3 could be obtained. The fastest and the slowest components were denoted 1 and 3 respectively. In the curves from the measurements over the tail, the initial uptake phase *1* was evaluated as in the normal rats, and phases 2, and 3 referred to the fastest and slowest disappearance components, respectively.

The values of the disappearance constants and the intercepts on the ordinate axis after extrapolation of the different phases to zero time are presented in table VIII.

Table VIII Characteristics of radioactivity curves after intravenous administration of Radio-Hippuran in unilaterally nephrectomized rats. For definition of phases 1 2, and 3, and of the *k* and *a*-values, see table VI. Values after ± are standard deviations

	Phase 1		Phase 2		Phase 3	
	k	a_1	k	a_2	k_3	a_3
Tail blood sample curves	0.108 ± 0.027	15.7 ± 10.5	0.0142 ± 0.0018	12.3 ± 2.0	0.00141 ± 0.00045	2.2 ± 0.6
Thoracic blood sample curve	0.120	13.5	0.0232	11.5	0.00158	2.1
External measurements over the heart	0.175 ± 0.071	30 ± 5	0.0221 ± 0.0058	29 ± 4	0.00140 ± 0.00060	9.7 ± 2.3
External measurements over the head	0.159 ± 0.045	49 ± 22	0.0239 ± 0.0048	57 ± 9	0.00070 ± 0.00025	19.4 ± 3.6
External measurements over the tail	0.069 ± 0.025	89 ± 52	0.0174 ± 0.0035	71 ± 19	0.00063 ± 0.00040	41 ± 4

In comparing the k values of the blood sample and external measurement curves, it was found that a rather good agreement existed excluding k_1 of the curve from the external measurement over the tail. Some differences must, however be pointed out. The k_3 constant of the curves from the external measurements over the head and tail were significantly lower ($P < 0.01$) than k_3 of the other curves. The k_2 constant of the tail blood sample curve and external measurements over the tail showed lower values.

The parameters of the external curves showed higher values than those of the blood sample curves. The differences were most evident for a_2 and least for a_1. This discrepancy was enlarged the more peripherally the external measurement was performed.

Discussion

By mathematical interpretation of the radioactivity courses it is possible to make more precise quantitative comparison of changes in the disappearance of injected Radio-Hippuran in normal and uninephrectomized animals.

In unilateral nephrectomy the disappearance constants k_1 and k_2, showed generally lower values than in normal conditions.

The parameters a_1, a_2 and a_3 of the three phases in the external measurements increased in uninephrectomy. The ratio of parameters for the corresponding phase, external measurement to blood sample curve, showed almost the same values as in the normal condition. A tendency to a somewhat lower value of this ratio was, however evident upon comparison of the parameters a_2 and a_3. The importance of the divergence of the values of parameters in the external measurements and in blood has been discussed in Chapter VII and was attributed to a diffusion of Radio-Hippuran into the extracellular space.

Summary

1. The disappearance of Radio-Hippuran from blood in uninephrectomized rats was determined by blood sampling. A higher radioactivity level in blood was noticed in unilaterally nephrectomized than in normal rats.

2. The disappearance of the radioactivity from blood was found to be expressed as a tri-exponential function. The disappearance constants of the two phases with the fastest decreasing rates were found to be significantly lower than in normal rats. The parameters of the two phases with the slowest diminishing velocities showed significantly higher values than in normal rats.

3. External measurements over the heart and head resembled in shape the blood disappearance curve and were also found to be expressed as tri-exponential functions. The disappearance constants of the two fastest phases had lower values than those in normal rats, and the slowest phase was rather similar to that found in normal rats.

4. The external measurements over the tail had an appearance principally the same as in normal rats. These curves could graphically be evaluated in three exponential phases: one build-up phase and two disappearance phases. The constant of the fastest disappearance phase changed in a way similar to that of corresponding phase of the blood curve and of the externally obtained radioactivity curves over the heart and head.

5. The parameters of the phases in the

external measurement curves showed generally higher values than the corresponding parameters in the blood sample curves. A migration of Radio-Hippuran into the extracellular space seems to be the most probable explanation to these findings.

Part 5

Excretion of Radio-Hippuran through the kidneys

Urine sample curves

Urine samples were collected during 300 minutes in five rats and during 90 minutes in one rat after injection of Radio-Hippuran.

Result

The excreted amounts of radioactivity were, at four minutes, 8.06 ± 5.64 at 10 minutes, 30.76 ± 7.03 at 30 minutes, 54.52 ± 8.40 at 100 minutes, 71.53 ± 4.37 and at 300 minutes, 80.02 ± 5.22 per cent of the injected dose.

The urinary concentration of radioactivity expressed in per cent of administered radioactivity per minute is shown in fig. 22 A. The maximal excretion rate 4 7—8 3 per cent per minute, occurred at 3.5—5.5 minutes after injection of Radio-Hippuran. A continuous decrease of the urinary output rate was then noticed At 300 minutes, the urinary excretion rate amounted to 0 04 –0 01 per cent per minute

Radiocystogram curves

External measurement over the urinary bladder was performed in eight rats, two of which were killed t 30, and two at 100 minutes. The excreted radioactivity in urine in these four rats was determined by direct measurement of the removed urinary bladder In the other four animals, the total radioactivity eliminated was calculated from urine samples taken at about 100 minutes. The radioactive contents of the urinary bladder were then calculated in the eight rats from the cystogram curve as described in chapter VI. From the accumulation curve obtained in this way the concentration of radioactivity in urine was estimated.

Results

The total amount of radioactivity excreted in the urinary bladder t 100 minutes, calculated from urinary samples in four rats, was 87 0 ± 4.2 per cent. The radioactivity in the urinary bladder estimated from the radiocystogram curves as described above amounted to 24 7 ± 8.4 per cent at 4 minutes, 48.3 ± 7.2 per cent at 10 minutes, and 70 6 ± 5.3 per cent at 30 minutes. These values did not significantly differ from those found by direct measurement of the urinary bladder in the distribution studies.

The concentration courses of radioactivity in urine determined from the radiocystogram curves are shown in fig 22 B The maximal excretion rate amounting to 8.3—14.8 per cent per minute occurred at 2.5—6.0 minutes after the injection of Radio-Hippuran. Thereafter followed a phase of gradual decreasing rate, with a tendency to lower values of the urinary concentration at 100 minutes, compared with those of the urine sample concentration curves.

In comparing the k values of the blood sample and external measurement curves, it was found that a rather good agreement existed excluding k_1 of the curve from the external measurement over the tail. Some differences must, however be pointed out. The k_3 constant of the curves from the external measurements over the head and tail were significantly lower ($P < 0.01$) than k_3 of the other curves. The k_3 constant of the tail blood sample curve and external measurements over the tail showed lower values.

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Radiocystogram curves

External measurement over the urinary bladder was performed in eight rats, two of which were killed at 30, and two at 100 minutes. The excreted radioactivity in urine in these four rats was determined by direct measurement of the removed urinary bladder. In the other four animals, the total radioactivity eliminated was calculated from urine samples taken at about 100 minutes. The radioactive contents of the urinary bladder were then calculated in the eight rats from the cystogram curve as described in chapter VI From the accumulation curve obtained in this way the concentration of radioactivity in urine was estimated.

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Summary

1. The disappearance of Radio-Hippuran from blood in uninephrectomized rats was determined by blood sampling. A higher radioactivity level in blood was noticed in unilaterally nephrectomized than in normal rats.

2. The disappearance of the radioactivity from blood was found to be expressed as a tri-exponential function. The disappearance constants of the two phases with the fastest decreasing rates were found to be significantly lower than in normal rats. The parameters of the two phases with the slowest diminishing velocities showed significantly higher values than in normal rats.

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4. The external measurements over the tail had an appearance principally the same as in normal rats. These curves could graphically be evaluated in three exponential phases: one build-up phase and two disappearance phases. The constant of the fastest disappearance phase changed in a way similar to that of corresponding phase of the blood curve and of the externally obtained radioactivity curves over the heart and head.

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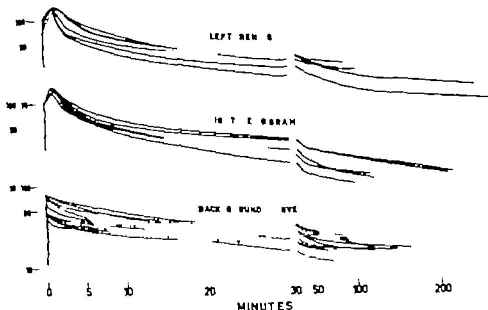


Fig 23. Radiorenogram curves over the remaining kidneys and background radioactivity curves over the region of the removed kidneys after intravenous injection of Radio-Hippuran in unilaterally nephrectomized rats. The dotted and continuous lines of the background curves refer to the right and left side, respectively

Radiorenogram curves

Six external measurements over each area of the remaining kidney under standard conditions fter injection of Radio-Hippuran were performed during 30—270 minutes. The radioactivity from the sur rounding tissues and organs causing the body background to the renogram curve was estimated by external measurements over the area of the removed kidney under standard conditions. Six body background curves were performed from each side during 30—210 minutes after injection of Radio-Hippuran.

Result

The radioactivity courses over the remaining kidneys are presented in fig 23. Their shapes were similar to those found in normal rats. When comparing the renograms from each side, tendency to higher values was found on the right side at one minute, and on the left side at 30 minutes.

The body background curves are present ed in fig 23 During the first ten minutes, there was an evident difference between the curves from each side. The right side curves started at significantly higher levels (1 one minute $P < 0.05$) and decreased faster than those on the left side. At 30 minutes the left and right-side curves agreed with each other in respect to their amplitudes.

Comparison of radioactivity in urine and in the remaining kidney

With the same purpose as in normal rats, the urinary excretion rate curves ob-

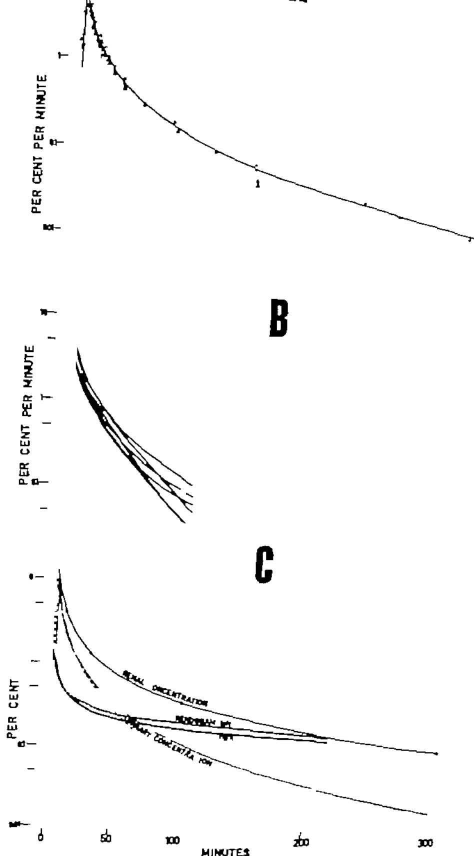


Fig 22. Urine radioactivity concentration curves estimated from urine sampling (A) and from external measurements over the urinary bladder (B) after injection of Radio-Hippuran into unilaterally nephrectomized rats. In C, the mean urinary output rate curves determined from the sample curves and radiocystograms have been plotted in the same diagram as the mean renograms and renal concentration curves estimated from the removed k...

Summary

1. The amounts of radioactivity excreted into urine after injection of Radio-Hippuran into uninephrectomized rats were estimated by urine sampling and by calculation from the radiocystogram curve.

2. The amounts of eliminated ^{131}I determined by radiocystography agreed well with the values found in the distribution studies by direct measurements of the urinary bladder.

3. The urinary ^{131}I concentration curve, obtained by urine sampling or estimated from the cystogram curve, indicated initially about half as large, and later a higher excretion rate, than in the normal condition.

4. A tendency to higher radioactivity was found in the uptake phase of the right renogram curve, and in the excretion phase of the left, when the radioactivity courses from each side were compared.

5. In unilateral nephrectomy the renogram curves lay at higher levels than in normal conditions.

6. External radioactivity measurements over the site of the removed kidneys revealed that these phenomena mentioned in 4 and 5 were mainly due to higher levels of the background curves.

7. The disappearance of ^{131}I from the remaining kidney had, during the first 30 minutes, nearly the same, and after 30 minutes, a slower diminishing rate in comparison with that of the urinary concentration curve.

Part 4

Excretion of Radio-Hippuran through the liver and external measurements over the thyroid gland

Bile sampling

The amounts of ^{131}I eliminated into bile were measured in five rats examined during 300 minutes. At the end of the experiments the livers of two rats were removed and measured for radioactivity.

Result

By 300 minutes a mean value of 2.45 ± 0.33 per cent of the injected radioactivity was recovered in the bile. The main portion of this amount was excreted during the first 60 minutes.

In fig. 24 the bile concentration is presented. A maximal excretion rate of about 0.08 per cent per minute at 5—10 minutes was observed, followed by a gradual decrease of the elimination rate. This diminishing of the excretion rate agreed during the first 75 minutes with that of the real liver and blood concentration and thereafter occurred somewhat faster than those.

The radioactivity of the liver at 300 minutes corresponded well to the values found in non-catheterized rats.

Radiohepatogram curves

External measurements over the right area of the liver in three rats in which the right kidneys were removed, were carried out during 140—210 minutes.

tained by urine sampling or calculated from the radiocystogram curves were compared with the mean values of the direct measurements of the remaining kidney (fig 22 C) During the first 30 minutes the three curves decreased at an almost equal velocity but deviated later due to a slower rate of the disappearance of ^{131}I from the kidney

Discussion

The radioactivity recovered in urine by the sampling technique was lower than when the whole urinary bladder was excised and checked in the distribution studies for radioactivity This discrepancy must, as in normal rats, have been due to faulty sampling technique, and was most marked at 4 10 and 30 minutes. The radiocystogram curve, however reflected very well the amounts of radioactivity in the urinary bladder

After unilateral nephrectomy the maximal excretion rate of ^{131}I was, as expected, only about half the value of that in normal rats This was compensated for by a higher excretion rate after 10—15 minutes in unilateral nephrectomy In this respect the urinary concentration curves obtained from sampling technique and from external measurement agreed with each other

Other investigators have, after unilateral nephrectomy in experimental animals, found reduction in clearance and Tm values of inulin and Diodrast, respectively (Braun Menendez and Chiodi, 1947 and Watschinger and Verner 1949) Their results are, however not directly comparable with those of the present investigation because of the different techniques used.

Since the radioactivity contents of the left and right kidneys agreed in the distribution studies, the differences between the right and left renogram curves at one minute were very probably due to discrepancy of the body background radioactivity

By the external measurements over the nephrectomized side it was established also that the body background radioactivity was higher on the right than on the left side at one minute, but at 30 minutes no difference was detected.

After unilateral nephrectomy higher amplitude of the renogram curves was recorded than in the normal condition. Thus, significant differences were found at one minute ($P < 0.001$) and at 30 minutes ($P < 0.05$)

Judging from the levels of the background curves in the normal condition and in unilateral nephrectomy the increased radioactivity of the renogram curves in the latter instance was mainly caused by the increase in the body background curves.

As the results of urinary excretion and renographic examinations did not differ in rats examined one and two days after nephrectomy an effect of operation on the results can be excluded.

The relation of urinary to real renal concentration of ^{131}I agreed in this experimental condition with that found in the normal rats.

In investigations on uninephrectomized rats the author has not found any changes of the distribution of ^{131}I labelled Diodrast due to effect of the compensatory hyperfunction until about 7 days after the operation (Magnusson, 1962 b) Thus any influence of compensatory hyperfunction on the results in the present investigation can be excluded

radioactivity into bile was observed, indicating that the liver is a competing organ as regards the excretion of Radio-Hippuran.

The hepatogram curve showed no uptake phase, but a disappearance component followed after the initial rise. The bile and real liver concentration curve had almost the same diminishing rate as that found for the blood radioactivity. From this, an excretion mechanism in the liver resembling filtration or diffusion process may be assumed. The appearance of a concentration gradient of 5.8, 3.7 and 2.0 at 10, 100, and 300 minutes, respectively between bile and whole blood curves, reveals, however that there must be an active uptake and excretion of Radio-Hippuran in the liver.

Summary

1. In unilaterally nephrectomized rats observed during 300 minutes, two to three times as large amounts of radioactivity than in normal conditions were excreted into the bile.

2. The shape of the bile concentration curve agreed with that of the liver and blood. The maximal excretion rate was observed during the first 10 minutes followed by a continuous decrease in concentration, the diminishing rate of which agreed fairly well with that of the liver and blood concentration curves.

3. The presence of a concentration gradient between bile and whole blood indicates an active uptake and excretion of Radio-Hippuran in the liver.

4. The hepatogram curve increased rapidly to a maximum, and decreased thereafter at a rate somewhat slower than that of the real liver concentration curve.

5. External as well as direct measurements of the thyroid showed that small amounts of radioactivity were accumulated in this gland.

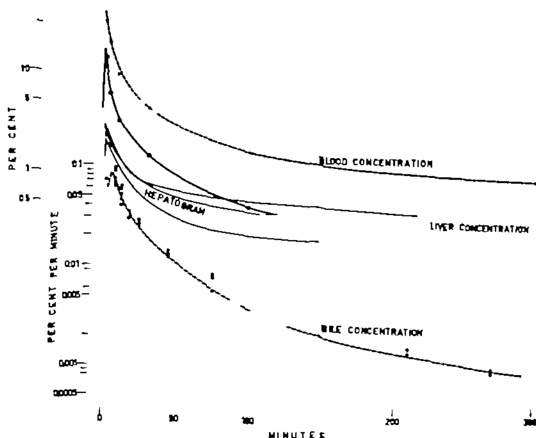


Fig 24 Time ^{131}I concentration curves in bile, liver and blood, and the hepatogram curves after intravenous injection of Radio-Hippuran in unilaterally nephrectomized rats. The right ordinate axis refers to the bile radioactivity concentration, and the left ordinate axis to the blood and liver radioactivity concentration. The hepatogram curves are expressed in relative counts per time unit and plotted in the same semilogarithmic system as the other curves.

Results

After a rapid increase of the radioactivity a falling phase was seen. The decreasing rate was somewhat slower than that of the real liver and blood radioactivity

External measurements over the thyroid gland

The radioactivity course in the thyroid gland was followed by external measurements in two rats during 220—240 minutes.

Results

A decreasing phase during the first 60—70 minutes and thereafter an uptake phase during the rest of the examination time were noticed.

Discussion

In conditions with decreased renal excretory power as after unilateral nephrectomy higher content of Radio-Hippuran in blood occurs resulting in accessibility of more radioactivity to the liver Hence as expected an increase of the excretion of

ical experiences from the use of radioactive iodide in the diagnosis of thyroid diseases (Skanse, 1949). In the present investigation, the amount of ^{131}I in the thyroid gland did not, however, differ from that found in normal rats during the first 300 minutes, but at 1 440 minutes more had been taken up than could be expected in the normal condition. The radioactivity of the urinary bladder showed a course similar to that of blood, which indicates that no accumulation of radioactivity occurred.

Summary

1. Studies on the distribution of injected Radio-Hippuran have been performed in bilaterally nephrectomized rats.

2. An evident retention of the radioactivity was observed in blood, from which ^{131}I disappeared at a very slow rate. In most organs the radioactivity diminished after 4—10 minutes at a rate nearly similar to that in blood.

3. An excretion of ^{131}I in the stomach and small intestine, several times larger than in normal and uninephrectomized rats, was detected from 10—30 minutes after the injection of Radio-Hippuran.

4. An uptake of ^{131}I in the thyroid gland was found. During the first 300 minutes the accumulated radioactivity did not differ from the results in normal and uninephrectomized animals.

Part 2

Disappearance of Radio-Hippuran from blood

Blood sample curves

Five bilaterally nephrectomized rats were injected with Radio-Hippuran, and blood samples taken during 300 minutes.

Results

The radioactivity of the total blood volume obtained by sampling from the tail as well as the results of thoracic blood measurements in the distribution studies are shown in fig. 23 A.

The first tail blood samples taken from two rats at 0.90—2.00 minutes contained radioactivity which corresponded to 28—36 per cent of the injected dose. A continuous diminishing of the disappearance rate was noticed during the first 20—30 minutes, followed by a constant exponential decrease of Radio-Hippuran during the rest of the examination time. In the other three rats, the first blood samples contained small amounts of ^{131}I, which was 17—21 per cent of given dose. Then the blood radioactivity showed a tendency to increase during the first 4—10 minutes, but after this time it agreed well with those of the other two rats.

During the first 20 minutes the blood sample curves were lower than those obtained in the distribution studies.

External scintillation measurements over the heart, head, and tail

Five radioactivity tracings over each of the three regions, representing various circulatory conditions, were carried out during 120—300 minutes after injection of Radio-Hippuran. At the end of the Radio-

CHAPTER IX

Behaviour of Radio-Hippuran in bilaterally nephrectomized rats

Part I

General distribution

One day after bilateral nephrectomy rats were subjected to distribution studies after injection of Radio-Hippuran. The radioactivity in organs which were assumed to accumulate, excrete, or metabolize Radio-Hippuran was determined in four anesthetized rats killed at each of the following time intervals after injection 1 4 10 30 100 and 300 minutes, and in two nonanesthetized rats killed at 1 440 minutes. Other organs and tissues were examined in two rats at the seven intervals above mentioned.

Results

The radioactivity of the different organs and tissues is given in table IX.

In most organs the radioactivity diminished at a rate similar to that in the blood. In some, this decrease occurred after an interval of a slight increase during the first 4—10 minutes. The radioactivity in the walls of the stomach and of the small intestine and in the thyroid gland tended to increase during the first 300 minutes. At 1 440 minutes an evident uptake of ^{131}I in the thyroid gland was noticed. There was also a large excretion of radioactivity into the stomach and the small intestine, and very small amounts were excreted into the coecum and colon.

Discussion

The results of the distribution studies revealed that a marked retention of Radio-Hippuran occurred in the blood at all time intervals as compared with normals, and at 10 30 100 and 300 minutes, as compared with unilaterally nephrectomized rats ($P < 0.001$) In bilateral nephrectomy the principal excretion of radioactivity occurred into the gastrointestinal tract, mainly the stomach and the small intestine. Since these parts of the gastrointestinal tract excreted large amounts of ^{131}I the possibility that the increase of the radioactivity in their walls had been caused by contamination of the excreted ^{131}I cannot be excluded.

It was expected that the uptake of ^{131}I in the thyroid gland should increase in ceased kidney function according to clin-

Spleen	0.43 0.30	0.36 0.30	0.30 0.25	0.36 0.27	0.27 0.20	0.20 0.18	0.12 0.14
Gl. parotis	0.41 0.49	0.39 0.33	0.26 0.24	0.21 0.22	0.17 0.17	0.16 0.18	0.13 0.08
Adrenal glands	0.070 0.068	0.077 0.065	0.058 0.038	0.047 0.042	0.076 0.034	0.040 0.046	0.033 0.036
Lungs	2.05 1.73	1.87 1.53	0.95 1.14	0.84 0.82	0.71 0.85	0.63 0.86	0.54 0.37
Uterus	0.750 0.856	1.078 0.616	0.975 0.516	0.523 0.267	0.467 0.432	0.363 0.356	0.189 0.196
Ovaries	0.117 0.142	0.134 0.136	0.110 0.091	0.069 0.085	0.091 0.082	0.089 0.070	0.080 0.046
Thymus	0.198 0.139	0.159 0.210	0.165 0.110	0.132 0.152	0.121 0.122	0.110 0.081	0.096 0.064
Skin (per gram)	0.18 0.25	0.77 0.62	0.68 0.56	0.64 0.67	0.53 0.57	0.49 0.55	0.46 0.27
Skeletal muscle (per gram)	0.32 0.18	0.27 0.23	0.27 0.25	0.23 0.22	0.22 0.21	0.24 0.24	0.19 0.13
Pancreas (per gram)	1.11 0.99	0.85 0.90	0.63 0.59	0.52 0.75	0.55 0.51	0.47 0.45	0.57 0.63
Brai (per gram)	0.151 0.106	0.106 0.105	0.077 0.093	0.060 0.078	0.049 0.060	0.050 0.068	0.068 0.043

Table IX Content of radioactivity in various organs of bilaterally nephrectomized rats, 1 4 10 30 100 300 and 1 440 minutes after intra venous injection of Radio-Hippuran. The values show the percentage of the given dose and standard deviations

Organ	1 minute	4 minutes	10 minutes	30 minutes	100 minutes	300 minutes	1 440 minutes
Urinary bladder	0.14±0.11	0.08±0.01	0.11±0.04	0.07±0.04	0.08±0.03	0.07±0.01	0.45
Blood	41.61±3.86	34.08±5.93	26.09±3.01	20.24±2.42	18.45±1.78	15.45±1.54	10.90 8.08
Thyroid gland	0.14±0.04	0.14±0.10	0.17±0.08	0.31±0.24	1.37±0.92	0.94±0.48	7.89 7.99
Liver	12.88±2.03	9.69±1.48	7.51±1.87	7.59±1.13	5.68±0.67	4.12±0.55	2.97 2.38
Stomach wall	1.06±0.22	1.08±0.23	1.14±0.34	1.00±0.24	1.27±0.22	1.60±0.46	2.02 2.93
» contents	0.29±0.05	0.48±0.10	0.67±0.34	1.46±0.46	3.01±1.42	3.55±1.21	9.03 22.30
Small intestine wall	4.36±0.66	3.80±0.25	3.36±0.54	4.21±0.30	4.81±0.87	5.98±1.53	4.43 3.21
» » contents	1.23±1.04	1.59±0.71	2.03±1.35	3.15±1.23	5.02±1.90	10.10±1.91	7.43 7.85
Coecum wall	0.96±0.76	0.60±0.07	0.61±0.15	0.50±0.12	0.79±0.46	0.74±0.21	0.93 1.31
» contents	0.34±0.33	0.28±0.17	0.26±0.14	0.25±0.11	0.71±0.18	1.00±0.37	4.88 10.11
Colon wall	1.30±0.17	0.98±0.22	0.84±0.10	0.97±0.32	0.80±0.17	0.52±0.25	0.50 0.79
» contents	0.19±0.16	0.15±0.12	0.14±0.12	0.10±0.03	0.37±0.18	0.64±0.40	0.16 0.14
Total gastrointestinal tract	11.83±2.92	8.98±1.50	8.90±0.94	11.64±1.39	16.80±2.48	25.52±0.55	35.54 52.23
Faeces							6.17 2.38

ated according to the principles described in Chapter VII.

Results

The tail blood sample curves from all rats, as well as the thoracic sample curve obtained in the distribution studies, could be resolved into two disappearance phases, 2 and 3. In the tail sample curves from three rats, there was also a build-up phase, 1.

Graphical evaluation of the curves from the external measurements showed that the heart curves in most cases could be divided into two disappearance phases, 2 and 3 sometimes third diminishing component, 1 was also detected. The head curves were composed of two disappearance phases, and the external tail curves could be divided into an uptake phase, 2, and a disappearance phase, 3. When two or three disappearance phases appeared, the slowest and the next slowest were always denoted 3 and 2, respectively

The values of the disappearance or build up constants and parameters of the different phases are presented in table X.

It was established that the k_3-values in all curves agreed well with each other as did also the k_2-values from the curves in which component 2 was a disappearance phase.

The parameter a_3 of all external measurements and a_2 in radioactivity tracings over the heart and head showed higher values than those of the blood sample curves. As in normal and uninephrectomized rats, the a_2 and a_3 parameters had higher values as the external measurements became more peripheral.

Discussion

The results of the measurements of tail blood samples, taken at different time intervals after injection of Radio-Hippuran, indicate, as in the distribution studies, a considerable retention of the administered radioactivity

In spite of totally abolished renal function, and no specific Radio-Hippuran ac

Table X. Characteristics of radioactivity curves in bilaterally nephrectomized rats after intravenous administration of Radio-Hippuran. Definitions of the phases 1, 2, and 3, and of k and a-values are given in table VI. Phase 1 of the tail blood sample curve and phase 2 of the curve from the external measurements over the tail, however were build-up phases. Phase 1 was not always obtained in this experimental condition. Values after ± give the standard deviations

	Phase 1		Phase 2		Phase 3	
	k	a_1	k	a_2	k	a_3
Tail blood sample curves	0.130 0.094 0.094	3.5 5.8 7.4	0.0383 ± 0.0136	7.8 ± 2.0	0.00049 ± 0.00023	21 ± 2
Thoracic blood sample curve			0.0450	2.3	0.00041	20
External measurements over the heart	0.360 0.213	1.2 5.0	0.0463 ± 0.0200	24 ± 10	0.00070 ± 0.00030	43 ± 3
External measurements over the head			0.0472 ± 0.0094	30 ± 12	0.00067 ± 0.00022	94 ± 4
External measurements over the tail			0.0141 ± 0.0068	215 ± 30	0.00036 ± 0.00020	269 ± 39

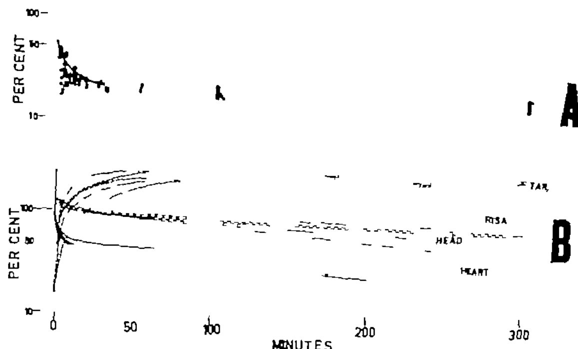


Fig. 25. Time-radioactivity curves in blood (A) and over the heart, head and tail (B) after intravenous injection of Radio-Hippuran in bilaterally nephrectomized rats. In B, the radioactive intensity of RISA is set at 100 per cent. In A, small dots and thin line represent tail blood samples, and big dots and heavy line thoracic blood samples.

Hippuran procedures, RISA was injected and the radioactivity tracings were continued for about another 10 minutes with the detectors in the same positions as during the previous checking. Moreover external measurement over the heart was performed in two rats, only after injection of Radio-Hippuran. These two curves were used in the estimation of the disappearance constants of the different phases.

Results

The radioactivity of Radio-Hippuran in per cent of that of RISA over the three parts of the body is shown in fig. 25 B.

Measurements over the heart. Immediately after the injection of Radio-Hippuran, a spike up to about 200 per cent followed by a decrease to 65—85 per cent was observed. The radioactivity curves then diminished during 15—20 minutes at a continuously reduced rate. A constant exponential disappearance rate then followed.

Measurements over the head. These curves increased rapidly to 100—130 per cent. Thereafter they decreased in a manner similar to the heart curves at a continuously reduced velocity during the first 20 minutes, and then at a more constant exponential rate. In two of the curves there was a tendency to increase during the first 0.5—1 minute.

Measurements over the tail. During the first 90—150 minutes the radioactivity courses showed an uptake phase with maxima of 205—265 per cent at 90—150 minutes, then tended to decrease slowly.

Interpretation of the radioactivity curves

The blood sample curves, as well as the external tracings, were graphically evalu

ated according to the principles described in Chapter VII

Results

The tail blood sample curves from all rats, as well as the thoracic sample curve obtained in the distribution studies, could be resolved into two disappearance phases, 2 and 3. In the tail sample curves from three rats, there was also a build-up phase, 1

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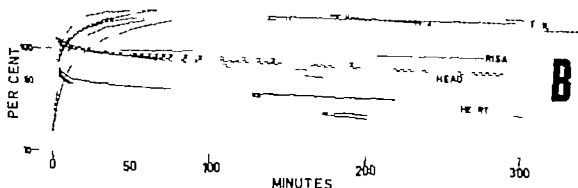


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in normal and unilaterally nephrectomized animals. The cause of this may be more complete mixing of Radio-Hippuran between the two distribution compartments, the blood and the rest of the extracellular and intracellular space.

Part 5

External measurements over the renal loins and over the urinary bladder

External measurements over the renal areas

External measurements over the places of the removed kidneys were carried out during 150—270 minutes after the injection of Radio-Hippuran. Five examinations were made over each side.

Results

The external measurements are shown in fig. 26. After a rapid increase immediately after the injection of Radio-Hippuran, the curves on the left side diminished during the first 2—3 minutes, and those on the right, during the first 4—8 minutes. Most of the curves then rose during the rest of the examination time. Two curves from the right, and one from the left side, reached a maximum at 30—150 minutes. The right-side curves started at a somewhat higher level than those on the left. The difference was significant ($P < 0.01$) at one minute. A tendency to higher radioactivity on the right side was also seen at 30 minutes.

Radiocystogram curves

Three measurements over the urinary bladder during 210—220 minutes after injection of Radio-Hippuran were carried out.

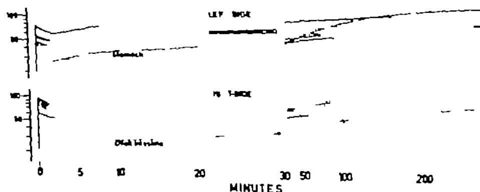


Fig. 26. Time-radioactivity curves of the backgrounds checked over the region of the removed kidneys after intravenous injection of Radio-Hippuran in bilaterally nephrectomized rats. The radioactivity of the stomach and the small intestine has been plotted in the same diagrams.

cumulation, excluding the gastrointestinal tract, which had a relatively low uptake, only a small portion of injected Radio-Hippuran was recovered in the blood. The labelled compound must therefore have been distributed in a space outside the blood. Since no firm binding of Radio-Hippuran to the serum proteins exists, as was shown in Chapter V Radio-Hippuran can easily move out into the extracellular space which was about 17 per cent of the body weight according to Wang and Hegsted (1949) If the blood occupies 7 per cent of the body weight, and the hematocrit is 40 per cent, the red blood cells should amount to 2 8 per cent of the body weight. Thus, the extracellular space and the red blood cells will together be 17 + 2.8 = 19 8 per cent of the body weight. If Radio-Hippuran were distributed, at the same concentration, in the red blood cells and the extracellular space, the whole blood should contain about 35 per cent of the injected dose, and this should also be the expected value of the a_1 parameter The value of the a_1 parameter obtained was, however 20—21 per cent. Radio-Hippuran therefore must either be taken up intracellularly in relatively large amounts, or exist in the extracellular space at a higher concentration than in blood. Since no proofs or reasons for the last mentioned hypothesis seem to exist, the first hypothesis is the most likely

In unilateral nephrectomy the ratios between parameters for corresponding phases in external measurements and blood sample curves decreased in comparison with the conditions in normal rats. In bilateral nephrectomy these ratios were further depressed for phase 3 A decrease of the gradient between the ^{131}I content in extracellular and intracellular spaces and in blood might have caused this phenomenon owing to more complete mixing between the two compartments.

Summary

1 A considerable retention of Radio-Hippuran occurred in the blood of bilaterally nephrectomized rats.

2. The blood sample curves could be expressed as a multiple exponential function with the regular appearance of two disappearance phases. From the results of graphical evaluation of the blood disappearance curves, the conclusion can be drawn that Radio-Hippuran is diluted or taken up in both the extracellular and intracellular space.

3 The external measurement curves over the heart and head had a shape similar to those in the normal condition and in uninephrectomy The curves could regularly be divided into two exponential disappearance phases.

4 The external measurements over the tail showed an uptake phase during 90—150 minutes and thereafter a phase with very low decreasing rate. This curve could be expressed as a two-exponential function with one build-up phase and one disappearance phase

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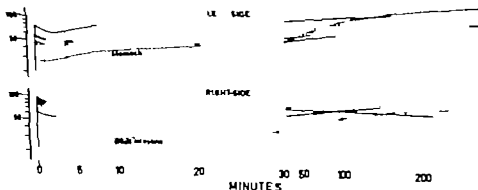


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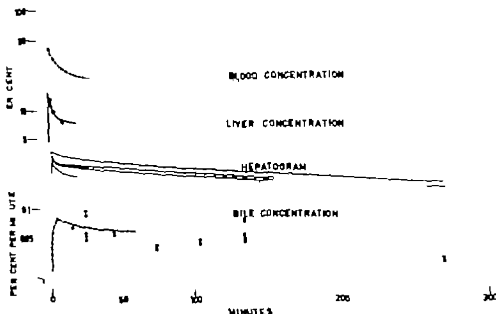


Fig 27 Time-radioactivity curves of radioactive content in blood, liver and bile, and hepatogram curves in bilaterally nephrectomized rats after intravenous injection of Radio-Hippuran. The hepatogram curves are expressed in relative counts per time unit and plotted in the same semilogarithmic system as the other curves in this figure.

Results

On the verage, 15.33 ± 4 77 per cent of the injected dose was excreted into bile during 300 minutes.

The bile excretion rate is presented in fig. 27 showing maximal rate of about 0.08 per cent per minute at 0—10 minutes followed by rather constant level amounting to 0.06—0.04 per cent per minute. The disappearance rate of this curve was in good accordance with that of the real liver and the blood concentration curves. The radioactivity of the liver at 300 minutes did not differ from the values found in non-catheterized rats. The radioactivity in the wall and contents of the small intestine amounted to 4.61 ± 1 07 and 7 19 ± 3.63 per cent of given dose, respectively The total gastrointestinal tract, excluding the bile, contained 19.21 ± 5.07 per cent. These values did not significantly differ from those found in the distribution studies.

Radiohepatogram curves

External measurements over the right part of the liver were made in four rats during 170—270 minutes.

R sult

The four hepatogram curves are shown in fig. 27 The radioactivity rose rapidly up to a maximum, and decreased at a rate rather similar to, or somewhat faster than, those of the real liver and blood concentration.

Results

After an initial rise, a falling slope followed during the rest of the examination time.

Discussion

The radioactivity measurements over the renal areas after the removal of both kidneys represent the body background contribution to the renograms in the extreme condition with completely eliminated kidney function. When comparing the external tracings over the left side, and the radioactivity found in direct measurements of the stomach (wall and the gastric contents) at intervals (fig 26) it was found that, on the whole, both these time-concentration curves agreed with each other In the same manner the right-side external curves can be compared with the time radioactivity estimations of the small intestine (wall and contents) and hence a rather good accordance can be observed. A higher initial level of the right side external radioactivity curves than of the left side is, however most probably due to the influence of the liver radioactivity

In comparing the external kidney area curves in bilaterally nephrectomized animals with the external empty kidney loin curves in unilateral nephrectomy higher levels were found throughout the whole examination time in the former case. The discrepancy was more evident as the observation time was extended.

The results of the measurements over the urinary bladder were in accordance with the findings in the distribution studies that no important accumulation of radioactivity occurred in the urinary bladder

Summary

1 External measurements have been *performed over the site of the removed* kidney after injection of Radio-Hippuran into bilaterally nephrectomized rats. The tracings showed, apart from the initial rise, a falling phase from 3 to 10 minutes, followed by an accumulatory segment during the rest of the examination. On the right side, the external measurements, in the beginning seemed to reflect the radioactivity in the liver and thereafter mainly that of the small intestine. On the left side, the external measurements coincided with the radioactivity in the stomach.

2. External measurements over the urinary bladder after an initial rise, showed a decreasing radioactivity intensity indicating that no important accumulation of radioactivity occurred in the urinary bladder

Part 4

Excretion of Radio-Hippuran through the liver and external *measurements over* the thyroid gland

Bile sampling

The radioactivity excreted into bile was measured in five rats during 300 minutes. At the end of the experiment the radioactivity of the liver the small intestine as well as of the total gastrointestinal tract was measured in four rats.

4 The external radioactivity tracings over the liver increased rapidly to a maximum and thereafter decreased at a rate similar to that of the blood and liver radioactivity

5. The external measurements over the thyroid confirm the results in the distribution studies that the gland actively takes up ^{131}I.

External measurements over the thyroid gland

Three rats were used, and the external measurements performed during 125—300 minutes.

Results

An immediate rapid rise after the injection of Radio-Hippuran was followed by a slowly diminishing phase. After about 30—45 minutes there was an increase during the rest of the examination time.

Discussion

Zum Winkel and de Maria (1961) measured the bile excretion of ^{131}I by pouch technique in bilaterally nephrectomized rats during 120 minutes after injection of Radio-Hippuran (Abbott) and found that it amounted to about 4.4 per cent. Somewhat higher values were found in the present investigation at the corresponding time interval, about 6.6 per cent. The differences in the results may depend on different experimental technique.

The bile excretion of radioactivity in bilaterally nephrectomized animals was considerably larger than in normal and unilaterally nephrectomized rats. Thus, the examinations have revealed that the liver is an organ competing for the excretion of Radio-Hippuran but that this competition is of importance only in conditions with severely damaged kidney function.

In contrast to the conditions in normal and unilaterally nephrectomized rats, the bile concentration curve in bilateral nephrectomy following the initial rise, showed a rather constant level. As in normal and unilaterally nephrectomized rats, the bile excretion rate curve agreed fairly well with the liver and blood concentration curves.

Despite totally abolished kidney function and an unusually high concentration of Radio-Hippuran in blood, no accumulatory segment was detected in the hepatogram curves, which could be expected, if an active uptake of Radio-Hippuran occurred in the liver. The appearance of concentration gradients of 5.1, 5.8, and 4.1 at 10, 100 and 300 minutes, respectively, confirms the earlier results in normal and uninephrectomized animals that the liver actively takes up Radio-Hippuran.

The sum of the recovered radioactivity in the bile and the contents of the small intestine, or the bile and the whole small intestine, or the bile and the total gastrointestinal tract, in the catheterized rats exceeded the radioactivity values of the corresponding parts of the gastrointestinal tract in non-catheterized rats. Therefore, some radioactivity excreted through the liver must be absorbed in the small intestine.

Summary

1. Large amounts of ^{131}I—about 15 per cent during 300 minutes—were excreted into the bile of bilaterally nephrectomized rats injected with Radio-Hippuran.

2. The bile excretion occurred at a rather constant rate which, on the whole, agreed with that of the liver and blood concentration of ^{131}I.

3. The occurrence of a concentration gradient between bile and whole blood indicates that there was an active uptake and excretion of Radio-Hippuran in the liver.

Thus, five patients with normal kidney function (group I) three with unilaterally damaged kidney function (group II) and two with bilaterally severely damaged kidney function (group III) have been investigated with combined external radiorenography which is the author's modification of the technique of Taplin *et al.* (1956)

N turally other problems too, have been investigated in the clinical part, for instance, the influence which various amounts of free radioactive iodide might have on the kidney function test.

Part 1

Binding of Radio-Hippuran to serum protein and red blood cells

The affinity of Radio-Hippuran to different blood components was investigated with the same technique as in the animal experiments.

Investigation on serum protein *in vitro*

Serum with normal electrophoretical picture was mixed with Radio-Hippuran in a tracer and a carrier dose. The carrier mixture contained 10 mg ortho-iodohippurate per 100 ml serum. Both mixtures were allowed to stand at room temperature for 30—60 minutes and were then examined in way similar to that described in Chapter V Dialysis was performed for 48 hours, continued for another 24 hours after change of the buffer solution. Ultrafiltration was carried out for about 15 hours. In electrophoretic examinations two of the buffer solutions described in Chapter V were used a barbital buffer with pH of 8.6, and a Sorensen phosphate buffer with pH of 7 4

Migration of Radio-Hippuran int the red blood cells *in vitro*

A tracer dose of Radio-Hippuran was added to a freshly drawn whole blood sample (hematocrit 40 per cent) and the mixture gently shaken and stored at a temperature of + 37° C. Samples were removed during the first two hours and treated as described in Chapter V

The influence of the *in vivo* milieu

In one patient (case 10 hematocrit 36 per cent) after the injection of Radio-Hippuran, blood samples were withdrawn t intervals, and centrifuged in heparinized glass tubes as soon as possible after collection, and the radioactivity measured separately in plasma and in the red blood cells. A blood sample taken at 3 minutes after injection of Radio-Hippuran was allowed to coagulate spontaneously and the serum subjected to electrophoretic examination. The electrophoretic strips were then scanned for beta ray activity

Results

Bi ding of Radio-H ppuran t serum proteins i it o and in vivo

1 *Protein pr cipitation* The radioactivity of the precipitate amounted respectively to 1 1 and 0.5 per cent of the total radioactivity in the tracer and carrier experiments in vitro

CHAPTER X

Investigations on humans

Our knowledge of kidney function in man is still incomplete. In the last thirty years, the quantitative aspects of renal function in higher animals and man have been extensively studied with the clearance techniques according to Rehberg and van Slyke. Many important branch phenomena of kidney function have, thus, been made clear. One has to bear in mind that besides the water and osmoregulatory power of the kidney two further main areas in renal physiology stubbornly demand interest, namely the regulation of renal blood flow and the biochemical aspects of renal function. All phases of kidney physiology have to be considered in evaluating different types of examinations of kidney function.

When radioactive methods came into use in clinical study of renal function a number of technical and biological problems joined the earlier aspects. Some of these problems have been elucidated in the early investigations by Taplin *et al.* (1956) and the author of the present investigation has made an attempt to systematical analyses of the basic factors by experimental animal investigations, the need of which has been again stressed recently by Kimbel, who at the International Symposium on Radioisotopes in Clinic and Research in Bad Gastein (1962) declared that a thorough knowledge of the biokinetics of the radioactive renal contrast media was the prerequisite for the diagnostical use of such compounds. Moreover at the above mentioned meeting, the usefulness of tagged ortho-iodohippurate in clinical work was emphasized by Taplin *et al.* zum Winkel, Roth *et al.*, Scheer and zum Winkel, and Bianchi and Toni.

In the clinical part, the author of the present investigation has given special attention to the possibilities of diagnostical recording of the excretion of Radio-Hippuran from the body by combined external radiorenography. In addition, studies on the affinity of Radio-Hippuran to human serum proteins and red blood cells have been included.

In order to throw some light on the clinical problems involved the author *has selected a number of special* renal cases admitted to the Medical Clinic at Serafimerlasarettet. Patients presenting special clinical problems were subjected to combined radiorenography as well as a number of individuals admitted to the hospital for a general medical examination, which revealed normal renal function.

Thus, five patients with normal kidney function (group I) three with unilaterally damaged kidney function (group II) and two with bilaterally severely damaged kidney function (group III) have been investigated with combined external radiorenography which is the author's modification of the technique of Taplin *et al.* (1956)

Naturally other problems too, have been investigated in the clinical part, for instance, the influence which various amounts of free radioactive iodide might have on the kidney function test.

Part I

Binding of Radio-Hippuran to serum protein and red blood cells

The affinity of Radio-Hippuran to different blood components was investigated with the same technique as in the animal experiments.

Investigation on serum protein *in vitro*

Serum with normal electrophoretical picture was mixed with Radio-Hippuran in a tracer and a carrier dose. The carrier mixture contained 10 mg ortho-iodohippurate per 100 ml serum. Both mixtures were allowed to stand at room temperature for 30—60 minutes and were then examined in a way similar to that described in Chapter V Dialysis was performed for 48 hours, continued for another 24 hours after change of the buffer solution. Ultrafiltration was carried out for about 15 hours. In electrophoretic examinations two of th buffer sol tions described in Chapter V were used a barbital buffer with pH of 8.6, and a Sorensen phosphate buffer with pH of 7 4

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Results

Binding of Radio-Hippuran to serum proteins in vitro and in vivo

1 *Protein precipitation.* The radioactivity of the precipitate amounted respectively to 1 1 and 0.5 per cent of the total radioactivity in the tracer and carrier experiments *in vitro.*

2 *Dialysis* The ratios of serum to buffer Radio-Hippuran at 24 hours were 3.9 and 2.4 and at 48 hours 1 6 and 1.5 in the tracer and carrier experiments *in vitro* respectively At 48 hours, 2.2 and 1.2 per cent, and at 72 hours, 0.3 and 0 4 per cent of the radioactivity remained in the bags containing the tracer and the carrier doses of ortho-iodohippurate, respectively

3 *Ultrafiltration.* After 15 hours suction 6.5 and 4.3 per cent of the radioactivity respectively remained in the tubings in the tracer and carrier experiments *in vitro*

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Migration of Radio-Hippuran into the red blood cells in vitro and in vivo

At about 30 minutes after adding Radio-Hippuran to whole blood *in vitro* an equilibrium seemed to exist between the radioactivity in red blood cells and plasma. From this time on, about 22 per cent of the radioactivity in whole blood was recovered in the erythrocytes. The migration rate of Radio-Hippuran to reach this equilibrium amounted to about 8 per cent per minute.

The relation of the radioactivity in the red blood cells to that of the total blood sample *in vivo* increased during the first 25 minutes and remained then at a rather constant level with a mean value of 24 per cent, corresponding to a ratio red blood cells to plasma, of 0.43 at a hematocrit value of 45 per cent.

Discussion

The results of the investigations on the binding of Radio-Hippuran to human serum proteins *in vitro* and *in vivo* are in accordance with those found in the animal experiments (Chap. V) They also agree with the findings of Bennhold *et al.* (1950) who observed that test agents used in examination of the renal excretory power usually moved as unbound substances in free electrophoresis with barbital buffer and with the suggestions by Burbank *et al.* (1961) From the results *in vitro and in vivo the conclusion can* be drawn that Radio-Hippuran has the possibility to diffuse from blood into other body spaces. This fact is of importance in the interpretation of the results of the external measurements over various regions of the body

The result of the investigation on the *migration of Radio-Hippuran into red* blood cells *in vitro* and *in vivo* is in *agreement with that found in the animal* experiments. The findings in the experiments *in vitro* did not quite agree with those of Smith *et al.* (1945) who tested non radioactive ortho-iodohippurate, but agree rather well with those of Burbank *et al.* (1961) who used the ^{131}I labelled compound. The former and latter investigators found ratios, content of ortho-iodohippurate in red blood cells to that in plasma, of 0.49 and 0.29 respectively The examination of the uptake of Radio-Hippuran in red blood cells *in vivo* in blood samples drawn at 25—62 minutes, showed a higher ratio than was found *in vitro* but the fact that this ratio remained at a rather constant level indicates that no firm binding of Radio-Hippuran to the red blood cells existed.

Summary

1 The binding of Radio-Hippuran to human serum proteins *in vitro* has been examined by protein precipitation, dialysis, ultrafiltration and electrophoresis, and *in vivo* by electrophoresis. The investigations revealed that none at all or only very small amounts of Radio-Hippuran seem to be bound to the serum proteins.

2 The uptake of Radio-Hippuran into human red blood cells *in vitro* and *in vivo* was investigated. The experiments *in vitr* revealed an uptake of Radio-Hippuran in the red blood cells with a mean ratio, red blood cells to plasma, of 0.53. In the examination *in vivo* a rather constant value of this ratio, on the average, 0 43 was obtained.

Part 2

Clinical applications of combined radiorenography

Methodology

Solutions

All solutions were kept in the dark in a refrigerator at +2 C.

1 The stock solutions of Radio-Hippuran were diluted with physiologic saline so as to obtain a sol tion with 15—20 μC ^{131}I per 2 ml. The concentration of orthoiodohippurate amounted to 15—70 μg per 2 ml. The content of fraction A was between 90 and 96 per cent.

2. The stock solutions of RISA were diluted with physiologic saline so that they contained 5—10 μC ^{131}I per 2 ml.

Appar tus for xternal sci tillation measurements

The two pparatuses for external measurements described in Chapter III were used. The collimator inserts in the animal experiment were replaced by two others a 12° wide angle insert for radiorenogram and radiohepatogram curves and measurements over the heart, and a 34 wid angle insert for measurements over the urinary bladder one foot, and the head. The outer opening of the first insert was 110 mm from the surface of the crystal, and had a diameter of 65 mm. The collimating effect was checked with a point source of ^{131}I at various distances. Thus, plateau-shaped scan-curves were obtained. At distances of 5 10, and 15 cm these plateaus were 8, 10, and 12 cm in breadth, respectively The outer opening of the other insert was 85 mm from the surface of the crystal, and had a diameter of 94 mm. The plateaus of the scan-curves were 13, 17 and 21 cm in breadth at distances of 5 10 and 15 cm, respectively

Apparatus for radioactivity measurements of samples

Blood samples in amounts of 5 ml were measured in the low-background well-scintillation detector described in Chapter III

Urine samples were measured for radioactivity with Tracerlab P 20 BQG scintillation detector with 1 × 1 solid crystal, described in Chapter III. Urine, or dilutions thereof, and standards in amounts of 200 ml were kept in identical glass vessels, and placed 147 mm from the crystal in the symmetry line during the measurements.

2 *Dialysis* The ratios of serum to buffer Radio-Hippuran at 24 hours were 3.9 and 2.4 and at 48 hours 1 6 and 1.5 in the tracer and carrier experiments *in vitro* respectively At 48 hours, 2.2 and 1.2 per cent, and at 72 hours, 0.3 and 0 4 per cent of the radioactivity remained in the bags containing the tracer and the carrier doses of ortho-iodohippurate, respectively

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1440 minutes. When the radiocystogram curves were made, the external recording was interrupted at about 60 minutes to allow the patient to urinate. Thereafter the radioactivity tracing was continued with the detector in the same position as before the interruption to estimate the residual radioactivity in the urinary bladder. In one patient the radioactivity in the urinary bladder was determined with a standard of Radio-Hippuran, which was measured at the same distance from the crystal as was the bladder.

Injection technique and interpretations of the radioactivity curve

About 2 ml of solutions of Radio-Hippuran and of RISA were injected into a cubital vein. The injected dose was estimated either by weighing the syringe both before and after injection and comparing the weight to standard or by measuring the radioactivity of the syringe both before and after injection at a distance of 147 mm from the solid crystal of the apparatus for sample measurements. By the last mentioned method the relation of injected dose of Radio-Hippuran to that of RISA was estimated. The external Radio-Hippuran curves were interpreted as described in Chapter VI. Thus, the radioactivity level of RISA in the external measurements was denoted by 100 per cent. The amplitude of the Radio-Hippuran activity curve was correlated to the administered radioactivity of RISA. The ratio of the amplitudes of Radio-Hippuran and RISA was then estimated. Similar calculations were performed in the measurements of blood samples on the assumption that equal blood volume of the samples of Radio-Hippuran and RISA was measured throughout the whole examination. The ratio Radio-Hippuran to RISA can be shown to be valid for the Radio-Hippuran remaining in the whole blood volume.

Case reports

Group I Normal kidney function series

Case 1 D.H. Acute pleurisy and myocarditis.

S.L. 888/60.

An 18-year-old man with myocarditis and recurrent pleurisy of unknown origin. No history of renal disease. The urine did not contain protein or pathologic sediment. Serum creatinine amounted to 0.70 mg per 100 ml serum.

The patient was examined during the recovery period with external measurements over each kidney and over the liver area after injection of Radio-Hippuran. The urinary excretion of radioactivity was also determined.

Case 2 H.K. Cardiac neurosis.

S.L. 215/62.

A 20-year-old man was admitted because of attacks of precordial pain, and, on some occasions, slightly elevated systolic blood pressure. Most of his troubles were considered to be psychogenic. Kidney examination with intravenous urography showed normal conditions. All conventional laboratory examinations of the urine were normal, and serum creatinine amounted to 0.83 mg per 100 ml.

External radioactivity tracings over each kidney, heart, and foot were carried out after intravenous injection of Radio-Hippuran and RISA. The disappearance of radioactivity from blood was estimated by sampling technique.

Record number at the Medical Department of Serafimerlasarettet.

Procedure of clinical investigations

External measurements

The patients were generally examined on two occasions. On the first, radioactive tracings over each kidney or over one kidney and the liver were usually performed, in some cases combined with radioactivity measurements of urine. The patient was seated, leaning forward.

On the second occasion, one or two days later scintillation measurements were made over the urinary bladder heart head, or foot. Blood sampling and, in same cases, urine radioactivity measurements were also carried out. The patient was lying on his back. The tracings were usually recorded for about 60 minutes following the injection of Radio-Hippuran and continued for another 10—15 minutes on occasions when RISA was injected at the end of the examination.

Radiorenogram curves The radioactivity was registered with the scintillation detectors directed from behind to the kidney areas. The kidney localisation was determined by one of two ways. In the first, a preinjection of 3—5 μC Radio-Hippuran was given, after which the area of highest radioactivity was estimated Then the test dose of Radio-Hippuran was injected. In the second method the detectors were moved a small distance up and down about half a minute after the injection of the test dose of Radio-Hippuran. If higher radioactivity was recorded at some distance from the original position, that place was considered as representing the kidney tracings, and the scintillation detector was left in this position. In cases where the original adjustment had failed, the tracing was repeated to obtain the first part of the curve.

Radiocystogram curves Radioactivity over the urinary bladder was recorded with the detector in front directed somewhat downward in the median line, to a point 3—5 cm cranially to the symphysis, with the outer aperture 1—2 cm from the skin surface.

Radiohepatogram curves The radioactivity was registered with the scintillation detector in front, directed somewhat upwards to the costal margin in the medioclavicular line.

External measurements over the heart area The detector was held in front in the median line with the collimator close to the skin, and the cranial edge of the outer opening at *manubrium sterni*

External measurements over the head The detector was placed in front, the collimator 1—2 cm from the skin, and the caudal edge at the base of the nose

External measurements over the foot The detector was pointed from above, and the toes were put into the collimator opening

Blood sampling

Samples were drawn from a polyethylene catheter inserted into a brachial vein, and collected in heparinized glass tubes. 5 ml of the samples were transferred to plastic tubes and measured for radioactivity In the examination of one patient the blood samples were centrifuged as soon as possible after collection, and the radioactivity measured separately in plasma and the red blood cells. Serum for electrophoretic examination was taken from the patient.

Urine sampling

Usually the patients were allowed to urinate at about 60 and 120 minutes, in some cases also at 30, 180 300 540 and

1440 minutes. When the radiocystogram curves were made, the external recording was interrupted at about 60 minutes to allow the patient to urinate. Thereafter the radioactivity tracing was continued with the detector in the same position as before the interruption to estimate the residual radioactivity in the urinary blad der. In one patient the radioactivity in the urinary bladder was determined with a standard of Radio-Hippuran, which was measured at the same distance from the crystal as was the bladder

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The patients were generally examined on two occasions. On the first, radioactive tracings over each kidney or over one kidney and the liver were usually performed in some cases combined with radioactivity measurements of urine. The patient was seated leaning forward.

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Radiorenogram curves The radioactivity was registered with the scintillation detectors directed from behind to the kidney areas. The kidney localisation was determined by one of two ways. In the first, a preinjection of 3—5 μC Radio-Hippuran was given after which the area of highest radioactivity was estimated Then the test dose of Radio-Hippuran was injected. In the second method the detectors were moved a small distance up and down about half a minute after the injection of the test dose of Radio-Hippuran If higher radioactivity was recorded at some distance from the original position, that place was considered as representing the kidney tracings, and the scintillation detector was left in this position. In cases where the original adjustment had failed the tracing was repeated to obtain the first part of the curve

Radiocystogram curves Radioactivity over the urinary bladder was recorded with the detector in front, directed somewhat downward in the median line, to a point 3—5 cm cranially to the symphysis, with the outer aperture 1—2 cm from the skin surface.

Radiohepatogram curves The radioactivity was registered with the scintillation detector in front, directed somewhat upwards to the costal margin in the medioclavicular line.

External measurements over the heart area The detector was held in front in the median line with the collimator close to the skin, and the cranial edge of the outer opening at manubrium sterni.

External measurements over the head The detector was placed in front, the collimator 1—2 cm from the skin, and the caudal edge at the base of the nose

External measurements over the foot The detector was pointed from above and the toes were put into the collimator opening

Blood sampling

Samples were drawn from a polyethylene catheter inserted into a brachial vein, and collected in heparinized glass tubes. 5 ml of the samples were transferred to plastic tubes and measured for radioactivity In the examination of one patient the blood samples were centrifuged as soon as possible after collection, and the radioactivity measured separately in plasma and the red blood cells. Serum for electrophoretic examination was taken from the patient.

Urine sampling

Usually the patients were allowed to urinate at about 60 and 120 minutes, in some cases also at 30, 180 300, 540, and

the urine was determined. The urine from the right and left kidneys contained 595 and 410 milliosmols per liter respectively

After injection of Radio-Hippuran, radioactivity tracings were recorded over each kidney area, and over the heart. The radioactivity excreted into the urine was also followed.

Case 7 G.H. Malignant nephrosclerosis. S.L. 1833/61.

A 51-year-old woman with familial tendency to hypertension and a moderately elevated blood pressure for the last two years. She had, however a hypertensive retinitis of stages II—III according to the classification of Keith, Wagener and Barker Analyses of the urine revealed a slight pyuria but no bacteriuria. Aortography showed that the left renal artery was narrower than the right, and the left kidney had a reduced cortical layer Serum creatinine and endogenous creatinine clearance were respectively 1 15—2.28 mg per 100 ml serum, and 56 ml per minute.

External scintillation determinations were performed over each kidney area, urinary bladder heart, head, and foot, after injection of Radio-Hippuran and RISA. The disappearance of radioactivity from blood was also determined by sampling technique.

Case 8 R.H. Malignant hypertension. U.A.S. 2303/61

A 62-year-old man with impaired vision was shown t ha e a hypertensive retinitis of grades III—IV according to the classification of Keith, Wagener and Barker Further investigation revealed a moderately elevated blood pressure. X-ray examination of the heart and electrocardiography showed changes indicating hypertrophy of the left ventricle. A trace of protein was discovered in the urine. In quantitative estimation of the urinary sediment normal values were found. In a urinary concentration test a specific gravity of 1.028 was reached. Serum creatinine values were 1.3—1.5 mg per 100 ml, and the endogenous creatinine clearance, 83 ml minute. Intravenous urography showed normal conditions. Aortography revealed a stenosis to about half the normal diameter of the left renal artery *normal accumulation and excretion* were, however observed on both sides. An attempt to perform selective clearance examinations failed.

External measurements were carried out over each kidney and over the heart. Radioactivity excreted into urine was estimated in samples.

Group III Bilaterally severely damaged kidney function.

Case 9 O.N Malignant nephrosclerosis. S.L. 9/62.

A man, aged 55, was admitted because of an attack of pulmonary oedema. He had a moderately elevated blood pressure. A slight proteinuria was detected. X-ray examination of the heart showed enlargement of the heart, especially the left ventricle. Serum creatinine and endogenous creatinine clearance amounted to 4.3—4.0 mg per 100 ml and 13 ml per minute, respectively X-ray examination of the kidneys without administration of contrast medium revealed a right kidney smaller than normal, while the left kidney could not be distinguished.

The radioactivity tracings were performed over each kidney area, heart, and

This patient was kindly placed at my disposal from the Medical Department of Uppsala Akademiska Sjukhus

Case 3 I.C. Orthostatism.
S.L. 280/62

A woman, aged 41 was admitted because of attacks of unconsciousness in erect position. These troubles were suspected to be orthostatic. She had no history of renal disease and laboratory examination of the urine showed normal findings. Serum creatinine and endogenous creatinine clearance were respectively 0.74 mg per 100 ml and 85 ml per minute.

After intravenous injection of Radio-Hippuran and RISA external measurements were carried out over the urinary bladder and the head.

Case 4 M.P. Hematuria incerte causae.
S.L. 340/62

A woman aged 39 had for the last six years had repeated attacks of hematuria, fever and pain in the region of the right kidney. Examinations with intravenous urography aortography retrograde pyleogram, cystoscopy and ureteral catheterization failed to reveal the source of the hematuria. Urine analyses showed an intermittent appearance of large amounts of red cells. The kidney function was normal as judged from a serum creatinine value of 0.74 mg per 100 ml, and an endogenous creatinine clearance of 96 ml per minute.

The radioactivity tracings after injection of Radio-Hippuran and RISA were performed over each kidney area, and the heart. The blood radioactivity was followed by sampling technique.

Case 5 Y.K. Pulmonary tuberculosis and suspected systematic lupus erythematosus.
S.L. 788/60.

A 33-year-old man, who earlier had had an active pulmonary tuberculosis, at present in a stationary period, was now admitted because of high erythrocyte sedimentation rate, 130 mm per hour. Analyses of the urine revealed a microscopic hematuria. The kidney function was considered normal, with serum creatinine values of 0.70—0.95 mg per 100 ml. Aortography showed normal conditions. Further clinical investigations gave certain support to the suspicion of systemic lupus erythematosus.

After injections of Radio-Hippuran the radioactivity was checked externally over each kidney area, and over the liver. The excretion of radioactivity in the urine was determined by sampling technique.

Group II Unilaterally damaged kidney function

Case 6 E.B. Leftside chronic pyelonephritis and diabetes mellitus.
S.L. 682/60

A woman, aged 62 with a benign diabetes mellitus for the last 21 years, was admitted because of pyuria and bacteriuria discovered at a routine control of her diabetes. Aortography combined with urography showed normal conditions in the right kidney but in the left, most of the cortical layer was destroyed. On this side there was also a considerably decreased capacity to accumulate and excrete the contrast medium. Retrograde pyelography showed normal anatomy in the left pelvis and ureter. The serum creatinine amounted to 0.93—1.33 mg per 100 ml and the endogenous creatinine clearance to 72 ml per minute. Urine from each kidney was sampled after ureteral catheterization and the power of each kidney to concentrate

the urine was determined. The urine from the right and left kidneys contained 595 and 410 milliosmols per liter respectively.

After injection of Radio-Hippuran, radioactivity tracings were recorded over each kidney area, and over the heart. The radioactivity excreted into the urine was also followed.

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A 51-year-old woman with familial tendency to hypertension and a moderately elevated blood pressure for the last two years. She had, however a hypertensive retinitis of stages II—III according to the classification of Keith, Wagener and Barker. Analyses of the urine revealed a slight pyuria but no bacteriuria. Aortography showed that the left renal artery was narrower than the right, and the left kidney had reduced cortical layer. Serum creatinine and endogenous creatinine clearance were respectively 1.15—2.28 mg per 100 ml serum, and 56 ml per minute.

External scintillation determinations were performed over each kidney area, urinary bladder heart, head, and foot, after injection of Radio-Hippuran and RISA. The disappearance of radioactivity from blood was also determined by sampling technique.

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A 62-year-old man with impaired vision was shown to have hypertensive retinitis of grades III—IV according to the classification of Keith, Wagener and Barker. Further investigation revealed a moderately elevated blood pressure. X-ray examination of the heart and electrocardiography showed changes indicating hypertrophy of the left ventricle. A trace of protein was discovered in the urine. In quantitative estimation of the urinary sediment normal values were found. In a urinary concentration test a specific gravity of 1.028 was reached. Serum creatinine values were 1.3—1.5 mg per 100 ml, and the endogenous creatinine clearance 83 ml minute. Intravenous urography showed normal conditions. Aortography revealed a stenosis to about half the normal diameter of the left renal artery normal accumulation and excretion were however observed on both sides. An attempt to perform selective clearance examinations failed.

External measurements were carried out over each kidney and over the heart. Radioactivity excreted into urine was estimated in samples.

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The radioactivity tracings were performed over each kidney area, heart, and

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head, after injection of Radio-Hippuran and RISA. The radioactivity in blood was measured by sampling technique.

Case 10 O O Chronic glomerulonephritis

S.L. 450/62.

A 41 year-old man had 12 years earlier had tonsillitis followed by an acute glomerulonephritis. Since then a slightly elevated blood pressure, and small amounts of proteinuria were observed. In connection with a syncope attack, the renal function was controlled. A serum creatinine concentration of 4 7—8 8 mg per 100 ml and an endogenous creatinine clearance of 11 ml per minute were detected. X-ray examination of the renal regions showed small kidneys. Less than 0.5 pro mille protein and no pathologic changes of the sediment were discovered. No bacteria were found in the urine.

Radiorenogram curves and external measurements over the head and urinary bladder were performed after injection of Radio-Hippuran and RISA. The amounts of ^{131}I excreted into urine was determined by sampling

Results

Normal kidney function series

Some of the results obtained from external measurements and blood sampling after injection of *Radio-Hippuran, with* a concentration of fraction A of 90—96 per cent, are shown in figs. 28 and 30

The disappearance of Radio-Hippuran from blood occurred at a continuously diminishing rate during the examination time, which was about 60 minutes. At 2 and 60 minutes about 40 and 2 per cent, respectively of the injected radioactivity were recovered in the whole blood volume.

The *radioactivity tracings* over the heart showed an initial spike up to about 180 and decline to about 90 per cent, then decreased at a gradually diminishing rate. On comparison with the blood disappearance curve, the external radioactivity curve lay at a higher level, and fell at a somewhat slower rate so that the radioactivity value at 60 minutes was 3—7 times higher than that in blood.

The external tracing over the head showed also an initial spike followed by a falling slope with a continuously decreasing rate. In addition, this curve showed also a higher radioactivity level at 60 minutes it was about 10 times higher than that of the blood sample curve

Over the foot, the radioactivity attained a maximal value during the first two minutes and diminished thereafter at a rate slower than was observed in the blood sample curve or in external measurement over the heart and head. The tail portion of the curve showed a value *16—17* times higher than was found in the blood sample curve.

The radiohepatogram curve reached a maximal value within half a minute and then fell at a rate similar to that in the external measurements over the heart and head.

The radiorenogram curves from both sides were identical. After an initial upswing they rose continuously and reached a peak at 3—4.5 minutes after the injection of Radio-Hippuran. Then followed a decline with a gradually decreasing disappearance rate between *3—5 and 30 minutes* after the injection, and thereafter a constant disappearance rate during the rest of the examination.

The radiocystogram curves began with an initial spike followed by a decrease

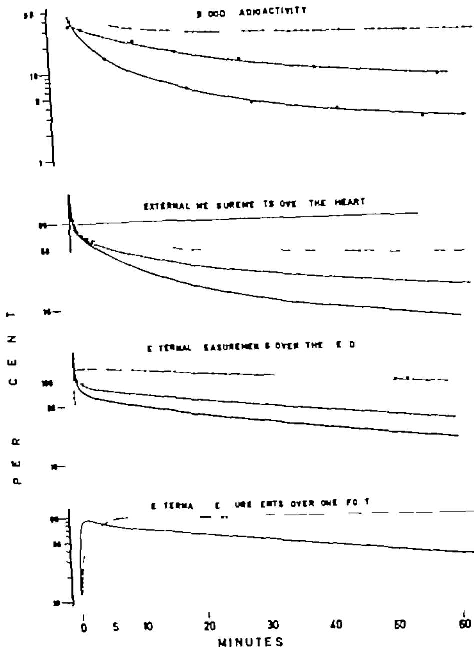


Fig. 28. Blood radioactivity and external measurements over the heart, head, and one foot after injection of Radio-Hippuran (containing 93–95 per cent fraction A) into four patients.
——— = normal kidney function (cases 2 and 3)
——— = suspected unilaterally impaired renal function (case 7)
= bilaterally severely damaged renal function (case 9)

head, after injection of Radio-Hippuran and RISA. The radioactivity in blood was measured by sampling technique.

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Radiorenogram curves and external measurements over the head and urinary bladder were performed after injection of Radio-Hippuran and RISA. The amounts of ^{131}I excreted into urine was determined by sampling

Results

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The radiocystogram curves began with an initial spike followed by a decrease

Severely damaged bilateral kidney function

The results of the radioactivity measurements in case 9 are shown in fig. 28.

In the two patients there occurred a marked retention of radioactivity in the blood e.g. at 60 minutes 15—20 per cent remained there. The blood disappearance curve showed less tendency to fall than was the condition in groups I and II.

The external measurements over the heart and head showed an initial spike followed by a disappearance phase. The descending rat of this component agreed rather well with that of the blood disappearance curve. At 60 minutes the relations of the radioactivity recorded over the heart to that in blood, and that over the head to that in blood were 2 and 4—5 respectively. The radioactivity tracing over the head started with a spike and a decline to 130—140 per cent. Then followed a decrease so that the Radio-Hippuran curve crossed the 100 per cent level at 35—45 minutes after the injection. In one patient the curve showed a slight tendency to increase during 2—3 minutes after the initial spike.

The radiorenogram curves in these patients resembled the radioactivity tracings over regions of the body mainly reflecting the blood and extravascular radioactivity disappearance. They fell, however at an even slower rate than that in blood or over the heart and head. From these results it may be suggested that the kidneys had no, or only very slight, ability to accumulate and excrete Radio-Hippuran.

The diminished excretory power was evident by urine sampling in case 10. Thus, at 60, 120, 180, 300, 540 and 1440 minutes, 25, 40, 49, 60, 72 and 84 per cent, respectively of the injected radioactivity was recovered in urine.

The radiocystogram curve from case 10 revealed that the excretion of ^{131}I began within the normal time, 3—4 minutes, but occurred at a very slow rate.

Discussion

Although most of the kidney function tests depend mainly on filtration or tubular transport rate, we must consider that these processes constitute only parts of the function of the whole organ.

In many aspects the kidney holds a unique position in the organism. It exists under a high oxygen tension maintained by a tremendous blood flow which normally amounts to about one-fourth of the total cardiac output at about aortic pressure. Despite the relatively small arteriovenous oxygen difference in comparison with many other organs, the total oxygen consumption of the kidneys is greater than that of any other organ in the body.

It is important to remember the fact that less than one per cent of the total renal energy production—as measured by its oxygen consumption—is used for the mechanical work, i.e. concentrating and excreting of the urinary constituents. The rest of the energy the kidney utilizes for its own internal biological processes which are not directly related to the external work. Still, we have incomplete knowledge of the internal renal metabolism. Only small alterations in the total renal oxygen consumption are found following considerable changes in the renal work. Thus, urinary formation and oxygen consumption by the kidney are not related phenomena.

Diodrast as well as Hippuran are eliminated from the blood by both glomeru-

both of which were assumed to be due to the body background radioactivity After 2—4 minutes a secondary uptake phase started, which represented the radioactivity excreted into the urinary bladder This phase had the steepest rise 6—8 minutes after the injection thereafter followed a steady diminishing of the accumulation velocity

The radioactivity excreted into urine amounted to 65—76 73—88, 82—92 and 84—95 per cent of the injected dose after 30 60 120 and 180 minutes respectively according to measurements of urine samples.

Unilaterally damaged kidney function

The results from the examination of case 7 are shown in figs. 28 and 30 and from the examination of case 6 in fig 29

The disappearance of Radio-Hippuran from blood determined by blood sampling revealed a retention of radioactivity as compared with the conditions in patients with normal kidney function. Thus, a radioactivity content about 2.5 times higher was found in case 7 at 60 minutes than was recovered in blood samples from cases 2 and 4

The radioactivity tracings over the heart and head had a shape similar to the corresponding curves in normal humans with an inital spike followed by a descending slope. In comparing the external measurements with the radioactive content in blood, the former showed higher radioactivity levels. The difference was more pronounced in peripheral regions of the body At 60 minutes, the radioactivity level over the heart and head was, respectively about 3 and 7 times higher than that in blood.

Over the foot the radioactivity increased slowly up to a maximal value of about 95 per cent at 8—10 minutes after the injection, and then decreased at a rate somewhat slower than that over the heart and head. At 60 minutes, the percentage level of Radio-Hippuran was 15 times higher than that of the blood sample curve.

The radiorenogram curves from the right side in these three patients had an appearance similar to that found in the patients with normal renal function. On the left side, however the shape of the radioactivity tracings differed in these three patients. In case 8 a radioactivity curve almost identical to that on the contralateral side and to the renogram curve found in normal kidney function was observed. In the left-side radiorenogram curves from cases 6 and 7 the initial upswing was immediately followed by a falling component, the disappearance velocity of which was considerably slower than that found in blood or external measurements over the heart or head.

According to the findings of the radiorenographic examination no ability of the kidneys to accumulate the radioisotope-labelled compound could be detected. The results obtained by radiorenography of the three patients were, thus, in good accordance with those found in radiologic investigations.

The radioactivity excreted into urine at 30 60 120 180 and 300 minutes amounted to 53—57 70—75 82—83 90 and 92 per cent respectively

The radiocystogram in case 7 showed that the excretion of ^{131}I began within the same time as did that of the normal subject examined (case 3)

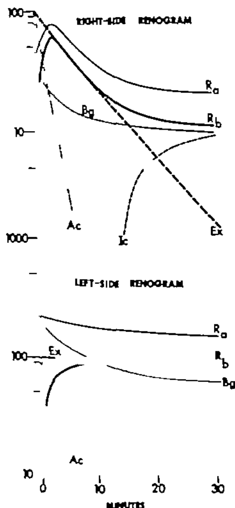


Fig 29. Semilogarithmic plotting of the radio-renogram curves from patient with left-side impaired renal function (case 6) The intercept of the *Ex*-line on the ordinate axis is set at 100. Explanation of the figure is given in the text.

In fig 29 the original radiorenogram curves (R_a) from the right and left side of a patient (case 6) with unilateral kidney function damage are drawn semilogarithmically Assuming that the initial upswing of the radiorenogram curve represents the first flood of the radioactive agent through the vascular bed of the kidney and the surrounding tissue, an externally recorded blood background curve was then attached to the top of this segment and subtracted from the renogram curve. The external radioactivity curve selected for representing this background was that recorded over the heart area (Bg) This subtraction resulted in a new curve (R_b) which theoretically ought to represent the real kidney radioactivity concentration. The excretion line (Ex) and the accumulation line (A) for the right normal kidney were constructed as mentioned above. Thus, the velocities, at which Radio-Hippuran was eliminated and incorporated, respectively could be estimated. It was noticed, moreover that with time there arose a deviation (I) of the R_b-curve from the Ex-curve. This can be explained in different ways, e.g. by a specific incorporation into the kidneys of Radio-Hippuran, or of radioactive metabolites not engaged in the urinary excretion. The deviation of the R_b and Ex-curves may also partly have been due to the excretion of Radio-Hippuran into the gastrointestinal tract, thus raising the body background curve with time.

When a renogram curve from a diseased kidney (the left kidney in fig 29) is graphically treated as mentioned above, the interpretation of the results is somewhat difficult. It can be seen that the slope of the *Ex*-line is very slight, with no significant deviation from the R_b-curve. An *Ac*-curve can easily be constructed. It is thus possible to perform the evaluation according to the principles previously adopted, but the meaning of the different constructed units may not be immediately evident. The result may indicate that the

lar filtration and tubular secretion. The molecules of the compounds are taken up by the tubular cells and transported through them finally to be expelled in the tubular lumen. This process consumes time, during which an accumulation of the substance in question takes place in the tubular cells. As soon as Taplin *et al* introduced the radioactive Diodrast kidney function test, the practical possibilities existed for measuring the tubular uptake-excretion process *in vivo* In this respect, *this new principle of testing the kidney function differed from other tests* previously used.

Attempts have been made by many investigators to evaluate radiorenogram curves after injection of various radioactive nephroattractive substances to gain quantitative informations about different qualities of the kidney function, *e g* glomerular filtration or tubular accumulatory and excretory abilities. The initial peak of the radiorenogram (the vascular phase) and the following slower increase (the accumulatory phase) were considered to be due to the arrival of radioactive blood in the kidney and a tubular accumulation of the radioactive compound, respectively At the maximum of the curve the uptake and outflow of radioactivity in the kidney are balanced. The following decrease in the radiorenogram (the excretory phase) has been regarded roughly as representing the urinary excretion of the test agent.

When performing radiorenogram curves after injection of ^{131}I-ortho-iodohippuric acid, Witcofski *et al.* (1961) used a high paper velocity of the recorder for the first 50 *seconds* (12 *inches* per minute) in order to be able to divide the initial vascular part of the curve into three parameters 1 the appearance time in each kidney 2, the time increment from the initial influx of radioactivity to the origin of radioactivity level 3 *the mean vascular* time. The rest of the curves was recorded with the slower paper velocity of 12 inches per hour The curves were plotted semilogarithmically and the different phases were evaluated as *mentioned* below External measurement over the *sternum* was assumed to record the disappearance of iodohippurate from blood. To get quantitative values from the blood curve, a blood sample was taken at the end of the investigation and checked for radioactivity The radioactivity excreted into the urinary bladder was also recorded from the outside and compared to a standard measured in a urinary bladder phantome.

Poker *et al* (1960) plotted the ^{131}I Diodrast renogram curves on arithmetic paper and evaluated the heights and areas under various segments of the curves. They also separated the curves into 1) a vascular phase, 2) a secretory or accumulatory phase, and 3) an excretory phase.

It has been valuable in many respects to plot radiorenogram curves semilogarithmically whereby the excretory phase usually could be drawn as a straight line. Graphically a secretory line could then be constructed by subtracting the accumulatory phase from the excretory Other characteristics of the radiorenogram curves have also been recorded from the graphical plotting

Irrespective of which evaluation method is used, the influence of the body background on the radiorenogram curves must be taken into account. Although this complicates the interpretation, it may be of both practical and theoretical interest to get an approximation of the renal concentration of *Radio-Hippuran.*

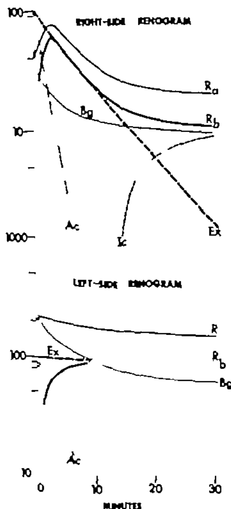


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ent investigation confirm earlier observations that [131]I-labelled ortho-iodohippurate has a superior specificity for renal excretion over other radiorenographic substances. The amounts excreted by 30 minutes in normal humans agreed well with the values found by Meade and Shy (1961) and Winter *et al.* (1961). In patients with severely damaged kidney function with a very low rate of urinary excretion of Radio-Hippuran, nevertheless, the main part of it would finally be eliminated through the kidneys.

The shape of the radiorenograms which reached a maximum level within 3 — 4.5 minutes was almost identical to that of the curves published by Nordyke *et al.* (1960) and Meade and Shy (1961).

Since the uptake of [131]I Diodrast in the liver often influenced the right radiorenogram, special interest has been directed to the possible uptake of Radio-Hippuran in this organ in humans. Measurements over the liver area in subjects with normal kidney function showed no accumulatory segment—the curve had the same form as those registered over the heart and head, and thus could be regarded as general "background" curve. These important circumstances were already postulated in the animal experiments.

Since final exponential phase was not recorded in the different radioactivity curves, no ample basis for the division of the curves in different exponential phases existed. The disappearance rate of the external measurements over the heart, head and foot, however seemed to be roughly the same as that of the corresponding blood sample curve in the three groups of patients. The percentage level of the curves f the external tracings differed from that of the blood sample curve, to principally the same degree as was found in the animal experiments. Thus, the radioactivity level in the external measurement over these last mentioned regions of the body exceeded that of the blood sample curve. This excess was larger as the recordings became more peripheral. As the renal function became more impaired, however the less pronounced was this excess. This fact was clearly predicted according to the findings in the animal experiments. The discrepancies between the curves may be explained as due to a diffusion of Radio-Hippuran into the extracellular space—a question of great theoretical and practical interest.

Human serum proteins, like those of rat serum, had no or only a very small affinity to Radio-Hippuran. Since the radioactivity courses in the external tracings exceeded those from the blood sampling by several times, most of the radioactivity recorded externally must have derived from Radio-Hippuran in the extravascular space. This means that there is no region in the body over which radioactive tracings can be performed with the hope of being able to measure the blood biokinetics of Radio-Hippuran during the whole experiment. After the first 15 minutes, the disappearance *rate* of the external radioactivity tracings, greed, however rather well with that of the blood sample curve, indicating the same diminishing velocity of the radioactivity from the extracellular room as from blood. It is notable that the external Radio-Hippuran curve over the head reached almost immediately such high values as 130—140 per cent in patients with severely damaged kidney function which may indicate that Radio-Hippuran easily passes through the capillary walls. Not until after 35—45 minutes did

tubules have ability to accumulate Radio-Hippuran, but not to excrete it. According to the *Ac* line, this tubular accumulatory power is fairly good when compared with that of the healthy kidney. On the *other hand, the Ac line might depend only* on the increase in the local body background. As the tracing for the diseased kidney is also lower than that for the normal, a decision on the different possibilities for an explanation must be rather speculative.

Evaluation of the radiorenogram curves according to the graphical principles outlined above may often be helpful, but in some instances it will produce confusing data. Therefore, a strict numerical graphical evaluation of the radiorenograms will *not give information which can be used* as a basis for quantitative estimation of the tubular function.

A somewhat uncertain point is the initial upswing of the radiorenogram which may be due to the radioactivity in the blood in the kidney and in the nearby big vessels, aorta and vena cava, if they also are exposed to the crystal. Some investigators have reported that the initial spike is not a good parameter of the blood flow in the kidney. Although the blood flow through the kidneys amounts to 25 per cent of the cardiac output, the blood content of the kidney is only 2—3 per cent *of the total blood volume* (Magnusson, 1960). As to the second, slowly increasing phase *of the radiorenogram, it is* obviously due to the concentration of the ra*dioactivity in the kidney. This can be due* either to a concentration of the glomerular filtrate through an absorption of water in the tubuli resulting in a lower urinary flow of radioactivity or to an accumulation of Radio-Hippuran in the tubular cells or a combination of both. Since the blood normally *is almost completely* cleared from the ortho-iodohippuric acid by its *passage through the kidney*, this indicates the tubular participation in the removal *process. Thus*, the second segment of the radiorenogram curve should be due mainly to the function of the tubular cells.

The third, decreasing segment of the radiorenogram curve must depend on the removal of radioactivity from the exposure of the crystal, which occurs when the radioactivity excreted into the tubular ducts is transported down into the urinary bladder, or could be explained by an absorption of radioactivity into the blood. The disappearance *rate visualized by the* slope of the third segment, thus, normally *depends both on the function of the tubular* cells, and also on the velocity of the urinary *flow which is governed by both the glomerular filtration* rate and the reabsorption ability of the tubular cells. The secretory and excretory phases of the radiorenogram curve thus should reflect both tubular function and glomerular filtration, the former may be the main regulatory factor under normal conditions. Therefore, the relative increment during the second phase of the radiorenogram curve is principally a measure of the tubular function of the kidney.

In cases with severely damaged kidney function the uptake and excretion of the injected ortho-iodohippurate in organs other than the kidney may interfere with the radiorenogram curve. This is clearly *pointed out in* the animal experiments, in which the injected substance was excret*ed in considerable* amounts into the bile and gastrointestinal tract of bilaterally *nephrectomized* rats.

The experiences in humans in the pre

7 Combined renal radiorenography—external tracings over each kidney urinary bladder heart or head—together with blood and urine radioactivity measurements, furnished the most relevant data on the biodynamics of Radio-Hippuran, and, thus, appears to provide a rather simple and rapid test of estimating the function of each kidney separately without the necessity for instrumentation of the urinary tract.

Part 3

Influence of varying amounts of $^{131}I^-$ in Radio-Hippuran on the radioactivity measurements

It has earlier been shown in this work that Radio-Hippuran contains a radioactive component, the behaviour of which is chromatographically electrophoretically and biologically identical to $^{131}I^-$. The content of this component some days after the delivery day amounted to 3—9 per cent, and on the storage of Radio-Hippuran under some unfavourable conditions, the concentration of $^{131}I^-$ sometimes reached values as high as 30 per cent. Some examinations were made in order to evaluate the influence which varying amounts of $^{131}I^-$ might have on the kidney function test with Radio-Hippuran.

Methodology

Solutions

a. An original preparation of Radio-Hippuran *containing 93—95 per cent of fraction A.*

b. The solution in a. with $^{131}I^-$ added so that the content of fraction A amounted to 83 per cent.

c. The solution in a. with $^{131}I^-$ added so that it contained 75 per cent of fraction A.

Radioactivity measurements

External measurements were carried out over the renal areas, heart, and head after injection of solution a. At 60—80 minutes either solution b or c was injected and the external tracings continued for another 60 minutes. Thereafter a dose of RISA was injected and the radioactivity levels over the heart and head, and in blood samples were recorded. When external measurements over the urinary bladder were performed, solution was injected one day after solution a. The radioactivity excreted into urine was estimated after the injection of each solution.

The curves were evaluated as described in Part 2 in this chapter

Patients

Three normal patients, cases 2, 3 and 4 and two patients, cases 7 and 8, with suspected unilaterally damaged kidney function were examined. Case 8 was injected with solutions a and b and the other cases with solutions a and c.

the down slope of the Radio-Hippuran curve in these patients cross the 100-per cent level. In patients with normal or moderately diminished kidney function this crossing occurred within one-two minutes.

As was shown in the animal experiments, the renogram curve reflects the changes in the concentration of radioactivity not only in the kidneys but also in the surrounding tissues. At the same time as the renal accumulation and excretion powers decrease, resulting in less radioactivity in the kidneys and in a retention of radioactivity in the body the radioactivity level of the background rises, so that the radioactivity registered over the renal area comes mainly from that of the surrounding tissues and organs. The possibility of estimating the remaining renal function from the external radioactivity tracings must therefore be limited under such conditions. This fact may explain why no accumulatory phase could be observed in cases 9 and 10 although an excretion of radioactivity to the urinary bladder existed. The results also demonstrate the value of radioactivity recordings over the urinary bladder

Summary

1 Radio-Hippuran was injected into patients with either normal or impaired kidney function. Some suffered damage mainly in one kidney while others sustained severe damage in both kidneys. External tracings of radioactivity were recorded over various regions of the body and radioactivity in blood and urine samples checked. The reference substance (RISA) was used according to the technique developed in the experimental studies.

2. The extreme specificity of Radio-Hippuran for urinary excretion was established. Even in a patient with severely damaged kidneys most of the injected dose was excreted into urine within 24 hours.

3 The radiocystogram curve furnished valuable information about the urinary excretion rate and the appearance time, i.e the time passing from the injection to the first appearance of radioactivity in the urinary bladder

4 Radiorenogram curves from patients with normal kidney function showed, after an initial upswing, an accumulation phase for 3—4.5 minutes, followed by an excretion phase. In some patients there was no accumulation phase registered over either one or both kidneys. In these cases, laboratory and different x ray examinations revealed severely impaired kidney function.

5 The influence of the background radioactivity on the radiorenogram curve limits somewhat the proper judging of the residual renal function in patients with severely damaged kidneys. This problem is pointed out and discussed and some methods of evaluating radiorenograms are proposed.

6 The external radioactivity tracings over the heart, head, and foot, had, after about 15 minutes, almost the same disappearance rate as that of the blood sample curve. The amplitudes of the external Radio-Hippuran curves lay consistently at higher levels than the blood sample curve The discrepancy was more pronounced as the external tracing became more peripheral. This held true for patients with normal as well as with damaged kidney function.

7 Combined renal radiorenography—external tracings over each kidney urinary bladder heart or head—together with blood and urine radioactivity measurements, furnished the most relevant data on the biodynamics of Radio-Hippuran, and, thus, appears to provide a rather simple and rapid test of estimating the function of each kidney separately without the necessity for instrumentation of the urinary tract.

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Results

The results of the investigations on the three patients with normal renal function and on case 7 are presented in fig. 50.

The blood radioactivity decreased slower after injection of solution c than after solution a.

The radiorenogram curves showed a tendency to flatten out after injection of solutions b and c. Thus, the initial rise and the uptake phase did not reach the same level as when solution was administered. When the Radio-Hippuran-Iodide mixture was administered, however the excretory component decreased slower. This resulted in a higher radioactivity level from 7—11 minutes than was the case when using of pure Radio-Hippuran.

Changes of the radioactivity courses similar to those found in the blood sample curves were also found in the external radioactivity tracings over the heart and head. Thus, the higher the content of $^{131}I^{-}$ in Radio-Hippuran, the slower the decreasing rate. By urine radioactivity estimations in patients 3 and 7 it was established that higher content of radioactive iodide in the Radio-Hippuran resulted in lower values of excreted radioactivity. In urine from cases 3 and 7 88 and 71 and 75 and 55 per cent were recovered at bout 60 minutes after injection of solution and solution c, respectively.

The radiocystogram curves had nearly identical shapes after injection of solutions a and b. This observation was made in the investigations on two patients, cases 3 and 7.

Discussion

Earlier investigations have shown that iodide is very slowly excreted into urine and taken up into the thyroid gland. Smith (1951) estimated the renal clearance at 31 ml per minute, while the thyroid clearance was even less. In the animal experiments of the present investigation it was shown that iodide had a slower kinetic turnover rate than ortho-iodohippurate, which fact agrees well with earlier investigations. Therefore, it must be suspected that the content of $^{131}I^{-}$ in Radio-Hippuran has a retarding effect both on the disappearance of radioactivity from blood and on its excretion into urine. This should be valid for animal experiments as well as for clinical investigations on man. The results of the present clinical investigation seem to verify this assumption.

When radioactive iodide was added to Radio-Hippuran, flatter renogram curves were consistently obtained. The tracings did not reach the same maximal value as when a pure Radio-Hippuran was injected. This was probably due to the fact that no uptake of iodide occurred in the kidney but only a filtration through the glomeruli. This agrees well with the results of the animal experiments in this investigation.

The elevation of the excretion phase of the renogram curve may be explained by the excretion of larger amounts of $^{131}I^{-}$ into the gastrointestinal tract, which causes high background to the radiorenogram.

Since the amounts of iodide excreted into urine during the first hour were very small as compared with the amounts of labelled ortho-iodohippurate eliminated, the radiocystogram curve mainly re

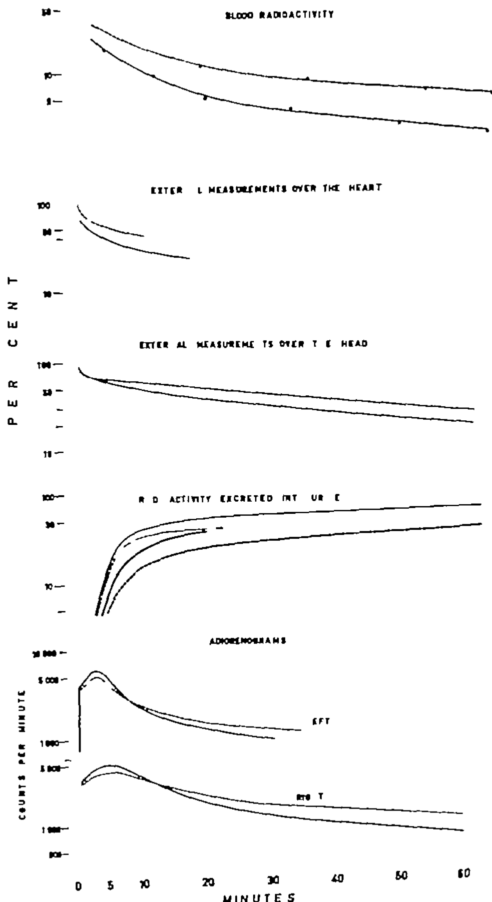


Fig 90. The content of 131I in blood and urine, and external tracings over various regions of the body (heart, head, left and right kidneys) after injection of two sol tions of Radio-H p-puran, one containing 93–95 per cent (whole line and dots) and another 75 per cent (dotted lines and half-filled circles) of fraction A. The radioactivity level obtained after injection of RISA is set at 100 per cent. The urine radioactivities were calculated from the radiocystogram curve Thin lines refer to examinations of patients with normal function (cases 2 3 and 4) and heavy lines to measurements on a patient with unilaterally damaged renal function (case 7)

Results

The results of the investigations on the three patients with normal renal function and on case 7 are presented in fig. 30.

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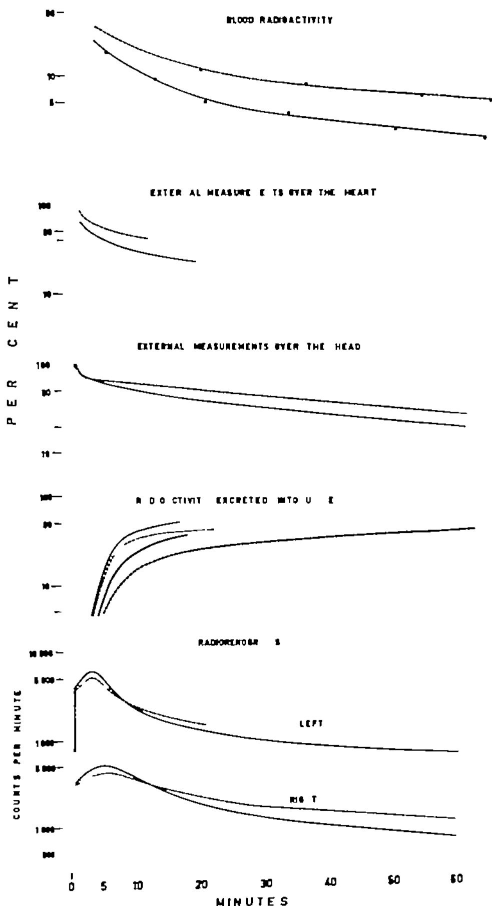


Fig 90 The content of ^{131}I in blood and urine and external tracings over various regions of the body (heart, head, left and right kidneys) after injection of two solutions of Radio-Hippuran, one containing 93—95 per cent (whole line and dots) and another 75 per cent (dotted lines and half-filled circles) of fraction A. The radioactivity level obtained after injection of RISA is set at 100 per cent. The urine radioactivities were calculated from the radiocystogram curve. Thin lines refer to examinations of patients with normal function (cases 2, 3, and 4) and heavy lines to measurements on a patient with unilaterally damaged renal function (case 7)

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Since the amounts of iodide excreted into urine during the first hour were very small as compared with the amounts of labelled ortho-iodohippurate eliminated, the radiocystogram curve mainly re-

flected the excretion of ortho-iodohippurate. This is perhaps the most probable explanation for the almost identical radiocystogram curves when Radio-Hippuran with varying amounts of $^{131}I^-$ was injected. The influence of the content of free radioactive iodide in Radio-Hippuran on the evaluation of the renal function is most clearly demonstrated by comparing the blood disappearance curves from cases 7 and 4 (figs. 28 and 30) Case 7 which had a moderately reduced renal function was injected with a "pure" Radio-Hippuran, while case 4 which had normal kidney function was injected with a mixture of Radio-Hippuran and iodide. In spite of the different renal excretory capacity in the two patients, the blood radioactivity curves showed nearly the same disappearance rates and radioactivity levels.

The influence of free radioactive iodide in preparations of ^{131}I-labelled ortho-iodohippurate on the results of estimations of the renal blood flow has been pointed out by Burbank *et al.* (1961)

The results in the animal experiments and in the investigations on humans showed that the occurrence of too high a content of free $^{131}I^-$ in the tagged ortho-iodohippurate can vitiate the proper judgement of the radiorenographic examinations. Therefore it is important to keep the content of $^{131}I^-$ in the preparations of Radio-Hippuran as low as possible. The results of the chemical analyses were such that some recommendations about the handling of Radio-Hippuran can be given. Since daylight and heat have a decomposing effect on Radio-Hippuran, storage of the labelled compound in brown-coloured glass bottles, or in the dark, and in a refrigerator is recommended. The addition of non radioactive sodium ortho-iodohippurate might decrease the autoradiolysis, but since no sterile solution of this substance is available on the market at present, the only way to reduce the autoradiolytic factor seems to be to dilute the stock solution.

In order to be able accurately to interprete the results of combined radiorenographic examination with Radio-Hippuran, the content of free $^{131}I^-$ in the solution must be estimated.

Summary

1 The influence of varying amounts of fraction B $^{131}I^-$ in Radio-Hippuran on the external measurements as well as radioactivity content of blood and urine was investigated in patients with normal and impaired kidney function.

2 The larger the amounts of fraction B —free radioactive iodide—in the Radio-Hippuran solutions, the more radioactivity was retained in blood. The changes in the radioactivity content in blood after injection of Radio-Hippuran with varying content of $^{131}I^-$ were also identical to those in the external measurements over the heart and head.

3 The percentage of radioactivity excreted into urine was lower after the administration of a Radio-Hippuran solution containing large amounts of $^{131}I^-$ than after injection of a pure solution of Radio-Hippuran

4 The semilogarithmic plots of the radioactivity excreted into the urine were almost identical after the injection of Radio-Hippuran with varying amounts of free radioactive iodide.

5 The radiorenogram curves were flattened out as the content of radioactive iodide

in Radio-Hippuran increased. The maximal value was depressed and the uptake and disappearance phases retarded.
6. Since the appearance of too large amounts of $^{131}I^-$ in Radio-Hippuran was disturbing factor in the clinical evaluation of the radiorenographic results, some recommendations as to the storage of Radio-Hippuran in order to minimize the decomposing rate of Radio-Hippuran are given. Thus, only small amounts of $^{131}I^-$ are released from Radio-Hippuran if it is stored in brown glass bottles, in the dark or in the cold. Dilution of the stock solutions of Radio-Hippuran to reduce the autoradiolytic effect is also recommended.

CHAPTER XI

General Summary

1 The purpose of the present investigation was to study the distribution and biokinetics of ^{131}I-tagged sodium ortho-iodohippurate in the organism in order to get information which could be used as the basis for quantitative evaluation of radiorenography

As some important data could most easily and in many instances only be obtained by animal experiments, the author has found it to be logical and necessary primarily to carry out such studies.

Earlier *Taplin et al. (1956) standard*ized the radiorenographic technique for different radioactive x ray contrast media. The author of the present investigation has modified this technique in view of the results of this experimental work. In order to elucidate the applicability of the method in clinical medicine, investigations on a number of selected patients *have been performed.*

2 Organic iodinated compounds have a tendency to decompose, and therefore a proper knowledge of the stability of a preparation of ^{131}I tagged sodium ortho-iodohippurate used in the present investigation, Radio-Hippuran (Abbott) is *essential in order to avoid misleading re*sults which will eventually depend on the decomposition products of the substance. By electrophoresis and chromatography it could be established that the main fraction of Radio-Hippuran, ortho-iodohippurate, actually disintegrates by splitting off free radioactive iodide. The liberation of iodide proceeded during storage but could be *retarded if the solutions* were stored in the dark, *e.g.* kept in brown glass bottles, or in the cold. The amounts of free iodide in the Radio-Hippuran solutions were never below 3.2 per cent of the total radioactivity Usually the free iodide amounted to 4—9 per cent. At these levels it could, however be established that the presence of free iodide in the Radio-Hippuran solution did not invalidate the usefulness of Radio-Hippuran for radiorenography When larger amounts of free iodide existed in the injected solution of Radio-Hippuran, it simulated a retarding disappearance of *Radio-Hippuran from* the body

3 Radio-Hippuran had no or very small affinity to human or rat serum *in vitro* as well as *in vivo* It migrated however rather rapidly into and out of the red blood cells.

4 In distribution studies on animals the extraordinary specificity of Radio-Hippuran for urinary elimination was established. Extrarenal uptake and excretion occurred exclusively into the thyroid gland and the gastrointestinal tract in

normal as well as in conditions with totally abolished renal function. Normally the competitory effect of the thyroid and gastrointestinal tract was minute, but the excretion into the gastrointestinal tract increased with the degree of renal failure.

In clinical investigations the extreme specificity of Radio-Hippuran for renal excretion was confirmed.

5. By direct radioactivity measurements of kidneys removed from rats, an accumulation of Radio-Hippuran was seen for the first minute followed by a continous decrease of the radioactivity at later time intervals. The external tracings over the renal areas reflected well the renal radioactivity concentration at the beginning of the recordings, but after 2—3 minutes the radiorenogram deviated gradually from the renal radioactivity concentration course owing to the influence of the radioactivity of the surrounding tissues. The background curve over the kidney areas was found to have different shape and amplitude on the left and right sides, and to increase in the same degree as the renal function decreased.

I the clinical studies the acquired knowledge of the experimental work was used in the interpretation of the radiorenogram curves. As to the radiorenogram, it was found that a disturbed kidney function could be recorded. In severely impaired kidney function the radiorenogram resembled the regional body background curve which was confusing as to the judgement of the remaining tobular function.

6. The radioactivity excreted into urine and estimated by calculation from the external scintillation recordings over the urinary bladder agreed well with the results gained by direct measurements of the removed bladders.

In clinical investigations the radiocystography was found to give valuable information about the urinary excretion rate and about the first appearance of radioactivity into the bladder

7 Radiochemical analyses of urinary excreted radioactivity indicated that the two radioactive fractions of Radio-Hippuran, ortho-iodohippurate and free iodide, were eliminated unchanged.

8. The urinary radioactivity concentration curve reflected well the changes in the renal concentration up to 30 minutes in normal and uninephrectomized rats.

9 In unilateral nephrectomy the maximal excretion rate of radioactivity into urine decreased in comparison with normally to about the half which was compensated for by higher urinary excretion rate at later time intervals.

10 A retention of radioactivity in blood corresponding to the degree of the renal impairment was observed in the experimental as well as in the clinical investigations. The disappearance curves in animals could be expressed as tri- and biexponential functions.

11 External radioactivity tracings over the heart, head and tail (foot in humans) showed, after dilution phase, a disappearance component. In the animal experiments, this component was composed of one two or three exponential phases, the disappearance rates of which agreed rather well with those of the corresponding phases of the blood sample curves.

In clinical investigations a fairly good agreement between the external tracings and blood sample curve was found concerning their disappearance rates.

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As some important data could most easily and in many instances only be obtained by animal experiments, the author has found it to be logical and necessary primarily to carry out such studies.

Earlier Taplin *et al.* (1956) standardized the radiorenographic technique for different radioactive x ray contrast media The author of the present investigation has modified this technique in view of the results of this experimental work. In order to elucidate the applicability of the method in clinical medicine, investigations on a number of selected patients have been performed.

2. Organic iodinated compounds have a tendency to decompose, and therefore a proper knowledge of the stability of a preparation of ^{131}I tagged *sodium ortho-iodohippurate* used in the present investigation, Radio-Hippuran (*Abbott*) is essential in order to avoid misleading results which will eventually depend on the decomposition products of the substance. By electrophoresis and chromatography it could be established that the main fraction of Radio-Hippuran, ortho-iodohippurate, *actually disintegrates by splitting off free* radioactive iodide. The liberation of iodide proceeded during storage but could be retarded if the solutions were stored in the dark, *e g* kept in brown glass bottles, or in the cold. The amounts of free iodide in the Radio-Hippuran solutions were never below 3 2 per cent of the total radioactivity Usually the free iodide amounted to 4—9 per cent. At these levels it could, however be established that the presence of free iodide in the Radio-Hippuran solution did not invalidate the usefulness of Radio-Hippuran for radiorenography When larger amounts of free iodide existed in the injected solution of Radio-Hippuran, it simulated a retarding disappearance of Radio-Hippuran from the body

3 Radio-Hippuran had no or very small affinity to human or rat serum *in vitro* as well as *in vivo* It migrated however rather rapidly into and out of the *red blood cells*.

4 In distribution studies on animals the extraordinary *specificity* of Radio-Hippuran for urinary elimination was established. Extrarenal uptake and excretion occurred exclusively into the thyroid gland and the gastrointestinal tract in

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12. The amplitude of the Radio-Hippuran curves recorded over the heart, head and tail (foot) was related to the radioactivity level after injection of ^{131}I labelled human serum albumin (RISA). This level was set at 100 per cent. The external Radio-Hippuran tracings had higher amplitudes than that of the blood sample curve. This discrepancy was most pronounced in the external recordings over the peripheral regions of the body.

Analyses of the blood sample and external tracing curves gave strong support to the existence of a migration of Radio-Hippuran into the extracellular and probably also into the intracellular compartments.

13. External scintillation measurements over the liver showed courses similar to those found over the heart and head. In bile, minute amounts of radioactivity were normally recovered but the excretion increased corresponding to the failure of the renal function. A concentration gradient between bile and whole blood indicated an active uptake and excretion of Radio-Hippuran into the bile.

14. The possibilities to estimate the different kidney functions in patients with various degree of damage of the kidneys have been critically and extensively discussed. So has also the significance with which a unilateral renal damage can be diagnosed.

15. The author of the present investigation has proposed a modification of the method of Taplin *et al.* for clinical investigation of kidney function with Radio-Hippuran. After intravenous injection of Radio-Hippuran radioactivity courses are recorded over kidney areas, and over heart, head and bladder regions. The blood radioactivity will be recorded by taking samples at intervals.

The radioactivity curves are then plotted semilogarithmically and evaluated taking into account thoroughly the changes in the biokinetics of the radioactive test agent exerted by disturbances in the kidney function.

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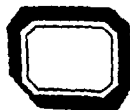
SICK ABSENCE IN A SWEDISH COMPANY

A SOCIOMEDICAL STUDY

BY

SVEN LOKANDER

MED. LIC.



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FROM THE MEDICAL DEPARTMENT OF ASEA, VÄSTERÅS (HEAD: DR ALLAN HEDFELDT) AND THE DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE, THE UNIVERSITY OF LUND, SWEDEN (HEAD: PROFESSOR GUNNAR LINDGREN, M.D.)

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ABBREVIATIONS

ASB = Sickness and Burial Insurance Fund at ASEA

BMI Blue Monday Index. *See page 14*

GSI = General Sickness Insurance

NN Local Temperance Board

SAS 00–316 Sick absence statistics definition number 00. *See page 11*

Diagnostic groups according to Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death

I Infectious and parasitic diseases
II Neoplasms
III Allergic, endocrine and metabolic diseases
IV Diseases of blood and haematopoietic organs
V Mental disorders
VI Diseases of the nervous system
VII Diseases of the circulatory system
VIII Diseases of the respiratory system
IX Diseases of the digestive system
X Diseases of the genito-urinary system
XI Diseases during pregnancy or parturition
XII Diseases of the skin
XIII Diseases of the skeleton and organs of locomotion
XIV Congenital disorders
XVI Symptoms, senility, ill-defined and uncertain diseases
XVII Injuries

INTRODUCTION

The state of health of an individual or of a group of individuals can be judged in different ways One method consists of analysis of the medical history and clinical examination This method is of great value to the individual. It may reveal pathological conditions and indicate what measures might be necessary to improve the person s state of health, and it might possibly yield information of the prognosis of any pathological conditions found In the estimation of the state of health of large groups of persons, however the value of the method is limited by the fact that it is difficult to draw a line of distinction between what should be regarded as normal and what should be regarded as *ill health*

Another way is to use the working capacity as a yardstick, ill health often resulting in a decrease in the output and precision of a person s work and in an increase in the number of mistakes he makes, etc. It is however as a rule, very difficult to assess to what extent the working capacity of a given person is decreased It is much easier to consider loss of working capacity only when it is so severe as to prevent a person from carrying on with his usual work. This applies, of course, only to individuals who are employed in companies keeping records of all absence because of sickness

Loss of working capacity due to ill health causes loss of income for the individual and his family as well as for his employer and, to some extent for the entire country

As far as the individual is concerned, a loss of income will vary with the type of disease, with the measures necessary to recover full working capacity with the duration of convalescence and with the amount of money he can expect from health insurance and from his employer

For the state and the community ill health of an individual implies loss of inland revenue and increased costs for the sickness insurance and for the medical care This is particularly important in Sweden, where the state is taking over an ever-increasing percentage of all costs of medical care

Sick absence implies both direct and indirect costs for the employer He thus has indirect expenses in the form of taxes and insurance fees and direct expenses in the form of sick pay and decreased output. Various attempts have been made roughly to estimate the effect of absence because of sickness on the balance sheet of industry Thus BOJER (1951) and ELFVENGREN (1954) assuming that the fixed costs of an industry are independent of the order of its output, found that such costs represented about 25 % of the turnover For the ASEA *Group* for example where the present investigation was carried out sick absence of 5 % would mean a loss of some 15 million Sw crowns In Sweden absence from work of a person for one day is usually regarded as incurring a loss of anything up to 100 Sw crowns for the employer According to this index, the absence among the 9000 persons employed in the ASEA in Västerås would have incurred a loss of

about 8.1 million Sw crowns. If the absence were the same for the entire group it would have involved a loss of about .5 million Sw crowns

The present investigation was started and carried out in the belief that it should be possible to find means of improving the general state of health of the personnel in the company and thus reduce sick absence and increase the output of the company That this is of interest t the personnel and to the company is obvious.

It was with the above purpose in mind that ttempts were m de to find answers to the following questions.

1 What is the order of the present absence because of sickness at ASEA in Västerås?
2 What diseases are responsible for most of the absence
3 What is the distribution of absence among the personnel?
4 What sources of error must be taken int account in the investigation f sick absence statistics f a company?
5 I it possible t exclude the distribution of sick absence being due to chance?
6 If the answer to question 5 is in the affirmative: in what respects do those who are often away from work because of illness differ from those who are seldom absent because of sickness?
7 Can the causes of such differences, if any between those who are often absent and those who are seldom absent be influenced by any means? In other words: Is it possible to ascertain whether some of the differences found are of environmental origin?
8. If the differences are found to be of environm ntal origin: can they be ascribed to working conditions, the way the individuals spend their leisure hours, to dietary (drinking) habits or to home environments during childhood or later?

In an attempt to answer these questions th investigation was carried out in a large company namely ASEA s f ct ry workshops and offices in Västerås

INTRODUCTION

The state of health of an individual or of a group of individuals can be judged in different ways One method consists of analysis of the medical history and clinical examination. This method is of great value to the individual. It may reveal pathological conditions and indicate what measures might be necessary to improve the person s state of health, and it might possibly yield information of the prognosis of any pathological conditions found In the estimation of the state of health of large groups of persons, however the value of the method is limited by the fact that it is difficult to draw a line of distinction between what should be regarded as normal and what should be regarded as ill health

Another way is to use the working capacity as a yardstick, ill health often resulting in a decrease in the output and precision of a person s work and in an increase in the number of mistakes he makes, etc It is, however as a rule very difficult to assess to what extent the working capacity of a given person is decreased It is much easier to consider loss of working capacity only when it is so severe as to prevent a person from carrying on with his usual work. This applies, of course, only to individuals who are employed in companies keeping records of all absence because of sickness

Loss of working capacity due to ill health causes loss of income for the individual and his family as well as for his employer and to some extent, for the entire country

As far as the individual is concerned a loss of income will vary with the type of disease with the measures necessary to recover full working capacity with the duration of convalescence and with the amount of money he can expect from health insurance and from his employer

For the state and the community ill health of an individual implies loss of inland revenue and increased costs for the sickness insurance and for the medical care This is particularly important in Sweden, where the state is taking over an ever increasing percentage of all costs of medical care

Sick absence implies both direct and indirect costs for the employer He thus has indirect expenses in the form of taxes and insurance fees, and direct expenses in the form of sick pay and decreased output Various attempts have been made roughly to estimate the effect of absence because of sickness on the balance sheet of industry Thus Bojer (1951) and Elfvengren (1954) assuming that the fixed costs of an industry are independent of the order of its output found that such costs represented about 25 % of the turnover For the ASEA Group for example where the present investigation was carried out sick absence of 5 % would mean a loss of some 15 million Sw crowns In Sweden absence from work of a person for one day is usually regarded as incurring a loss of anything up to 100 Sw crowns for the employer According to this index, the absence among the 9000 persons employed in the ASEA in Västerås would have incurred a loss of

about 8.1 million Sw crowns. If the absence were the same for the entire group, it would have involved a loss of about .5 million Sw crowns

The present investigation was started and carried out in the belief that it should be possible to find means of improving the general state of health f the personnel in the company and thus reduce sick absence and increase the output of the company That this is f interest to the personnel and to the company is obvious

It was with the above purpose in mind that attempts were made to find answers t the f llowing questions

1 What is the rder of the present absence because of sickness at ASEA in Västerås?
2. What diseases are responsible f r most of the absence?
3 What is the distribution of absence among the personnel?
4 What sources of error must be taken int account in the investigation of sick absence statistics f a company?
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CHAPTER I

STANDARDIZATION OF SICK ABSENCE STATISTICS AND MORBIDITY

It has long been desired to have norms for the statistical analysis of the state of health in a population. As early as 1855 the first international list of causes of death was presented at a congress in Paris (SJÖVALL 1951) This suggestion was not generally accepted, but it did stimulate further attempts at standardization, and in 1893 the first widely accepted nomenclature was agreed upon. This has often since been revised, and last time in 1948 under the direction of WHO The last revision was the most radical since it now also contains not only causes of death but is also to be used for morbidity statistics (SJÖVALL) The last revision, which appeared in 1957 contains only slight modifications compared with that of 1948 and it is now recognized as the international classification of diseases and injuries and causes of death The nomenclature on diseases is divided into 17 chapters which, with certain modifications, has been used by several investigators (e g MALAN 1958 SPRATLING et al. 1956 FORSSMAN 1958 1961 LINDGREN 1957 SUNDELL 1957) in the classification of diagnoses in the study of absence from work. As mentioned by SJÖVALL, the basis of the nomenclature is not uniform The nomenclature is based on a mixture of aetiological and special pathological principles. He nevertheless states that it is the best hitherto available

The method of keeping absence statistics has also been the subject of standardization, and in recent years this has resulted in an international classification In 1954 the General Register Office in England published "Measurement of Morbidity" and in Ontario in 1954 and 1955 the punch card system was recommended for large and small industries

In 1954 the Permanent International Commission on Industrial Medicine decided to have a conference on these problems It was held in 1957 in Leyden and its finally adjusted results were published in 1960 under the title of Sick Absence Statistics

Though no standardized definition were available before 1960 the following definitions are often encountered in the literature (FORSSMAN & VIGLIANI 1951 FORTUIN 1955 SVENNERUD 1959)

1 *Frequency rate* is the average number of absences per 100 or 1000 workers per day

2 *Disability rate* is the average number of days lost per worker per year

3 *Severity rate* is the average number of days lost by absence

Clear definitions were decided upon at the international conference in Leyden. Definitions of relevant interest are given below (Sick Absence Statistics Final Draft 1960) Hereinafter the definition will be referred to as SAS followed by the respective numbers

00 *Sick Absence* Absence from work accepted as attributable to sickness or injury

01 *Spell* A spell is an uninterrupted period f sick-absence

02 *Initial day* The initial day f a sickness spell is the first full day r substantial part of day a person is away from work.

03 *Final day* The final day of a sickness spell is.

a) the day preceding the day on which the person re mes work, either fully partly

b) the day on which the person absent dies,

c) the day before pensioning.

d) the day preceding th day of dismissal,

e) the 182nd day of the spell.

04 *A day f sick absence* is the initial day the final day or any f th calendar days lying between those days.

05 *Duration*. The d ration f a spell is the total number f days f ick absence belonging to a pell.

Measurements relating t spell observed within a gi en period f time

300 *The umbe of spell observed withi given period f time*

301 *The number f day f absence within a given period of time*

30? *The average umber f persons u der observation d ring a given period f time*

303 *The number of persons absent within a given period f t me*

304 *Man-days of observation*. This is the number of calendar days of the observation period multiplied by the average number of persons under observation.

310 *The period prevalence rate* This rate is the number of spells observed within a given period of time related to the average number of persons under observation.

311 *The average duration f completed r incompleted spells observed, per per son under observati n.* This rate is the number of days of absence related to the average numbe of persons under bservation.

315 *The proportion f persons absent in a given period f time* This rate is the number of persons absent within a given period of time related to the a erage number of person under observation.

316 *The proportion f persons never absent n a gi en period f t me*

Further recommendations

01 ige The age i defined as the differ ence between the date of the current year and that of the year f birth. The n mbers and rates hould alwa be calculated f some age groups separately These ge groups are recommended.

15— 4 years
—44
45—54
55—64
65—

OTHER DEFINITIONS

Here the term worker is to be under- tood an individual who receives hi wages once a week, and the term salaried employee an individual who receives monthly salary

Formerly the term worker was used to des gnate manual labourers and alaried employee was reserved for people not doing man al labou The border between these tw groups has however become

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CHAPTER II

PREVIOUS INVESTIGATIONS

Absence from work has been studied from various points f view These investigations have been made on the basis of data collected from industries, etc., where it has been possible to follow the development from one year to another Data from some of these companies are also collected at large centres, such as the N tional Institute for Preventive Medicine in Leyden, which every week recei es data from 70 industries with a total f som 9 000 employees This registration was started in 1947 (FORTUIN 1955 FORSSMAN 1958) In Sweden data on the absence for a certain week are collected twice a year by Socialstyrelsen (The National Social Welfare Board)

Thus in 1958 data were obtained from 1712 companies with a total of 381 000 male and 70 000 female salaried em ployees or workers. (*Sociala meddelanden* 1959)

Absence in general and particularly absence because of sickness is dependent on th st to f the health of the popula tion in general. It is therefore of great value to have access to the large compilations started in the USA, for example, where the N tional Health Survey commenced in 1956 to set up statistics on "disease, injury impair ment, disability and related topics on a uniform basis f r the nation as a whole" (Public Health Service Publication No. 584 1958.)

DISTRIBUTION OF ABSENCE AMONG EMPLOYEES

The distribution f ick absence both the number of occasions and the total number of days of absence in a popula tion, is not uniform. Thus GAFAFER (1913) found that 1 % f the men were responsible for 69 of the total duration of absence and of the women for 63 of the duration of absence. BEHREND (1951) tressed that th major part of the t tal duration f absence in a gi en company is deter mined by the absence of mi r group

In other words, some individual tend t be absent from work for relatively lo g periods per ar BEHREND (19 1), h wever warns against la ifying a man repeate until after several years of observation. LIDDELL (1951) found that the absence pattern of a given indi idual was as a rule the same f r three consecutive years, *i.e* those, who were never absent in the first year were also healthy the following years, while in dividual who were often absent one year were also often absent the following years etc Individuals wh are often absent are sometimes called "repeaters" and this group is f particular interest because it contains workers with a poor output and poor sense f respon ibility As support f this assumption reference has been made to HENRIKSSON s (1954) finding that salaried employees and w rkers who re often absent also often arrive late at their work. A for "repeat rs" FORTUIN (1955) pointed out that they should be di ided into 3 groups

1 Statistical repeaters. individuals who

more diffuse and is no longer valid Some authors have regarded workers as *people whose rates of pay were determined* by the trade unions and salaried employees as persons engaged on the basis of private agreements (see CRONER et al. *1959) It must be realized that both* groups are very heterogenous Such a division is hardly rational, and the difference between the conditions under which the two types are *employed* is becoming increasingly diffuse Nevertheless the differences between workers and salaried employees are still considerable regarding conditions on which they are employed and working conditions. This applies above all, to absence because of sickness. This point will be reverted to later

The wages of workers are calculated in different ways A worker may thus be employed on piece work, he may be paid by the hour he may be paid by the hour plus a bonus calculated on the basis of the output of a group of workers to which he belongs Sometimes the pay is calculated on the basis of the individual piece work, in other cases on the basis of the output of a team of workers group piece work Intermediate types may also occur One group of workers receives a *fixed* weekly wage. *These* are usually workers who have been in the company for a long time and rank between workers and foremen receiving a monthly salary *(As a sign of the* tendency of the difference between workers and employees to disappear it might be mentioned that from 1961 workers at ASEA receive their wages once a fortnight)

before and after a holiday with absence on other days

Hill (1929) in a paper referred to by Gordon et al., felt that the periods f illness hould be calculated in weeks rather than in days, since there is tendency for absences because of illness to start and end on the same day of the week. There i also aid to be a secondary monthly cycle. Sick absence also varies distinctly w th the season. Gafafer (1943) and de Groote (1955) tudied tha bsence for more than 10 years and found t regularly t be highest in the first quarter and lowest in the third. The variation was ascribable almost completely to respiratory tract diseases, while diseases of the digestive organs and other diseases showed hardly any variation at all. Henriksson (1954) found the frequency of absence to be highest in the Swedish material during the months of November—December and lowest during July—August. Norman et al. (1956) and the Health Statistics (1958) arrived at the same conclusion

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Behrend (1951) and Elfvengren (1953) reported a clear tendency for absence to be higher in larger companies. Henriksson (1954) however found no significant difference in his series of employees. Neither did he find the size of offices or workshops to have any ignificant influence on absence. For females working in groups f more than 70 absence was, however higher

REQUIREMENTS OF WORK

Regarding educational requirements see p 21

Some jobs require people with a high sense of responsibility others with a high degree of practical experience. Pugh et al. (1959) found that salaried employees in responsible positions had a low sick absence. In their in estigation it wa possibl t separate responsibility from educational level, which is otherwise not easy

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are absent on various occasions during the year for different unrelated diseases.

2 Justified repeaters, individuals suffering from a disease with recurrent symptoms

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According to FORTUIN the third group may be regarded as very small, and he feels that the total absence of workers and salaried employees in a given company cannot be decreased to any appreciable extent by trying to eliminate this group by disciplinary measures or other means

It is, of course not certain that an uneven distribution of absence because of sickness must be due to greater absence of a small group of employees and workers FORTUIN stresses that the distribution of absence because of sickness for a year closely resembles a random distribution according to Poisson's law If the period of observation however be extended to 5 years the distribution can no longer be ascribed to chance.

CALENDARIAL DISTRIBUTION OF ABSENCE

The calendarial distribution of absence is not uniform either Most authors have found wide fluctuations and most of them have also arrived at the conclusion that absence because of sickness is highest at the end or the beginning of a week-end, and lowest in the middle of the week BEHREND (1951) who focused special interest on voluntary absenteeism, introduced the term Blue Monday Index (BMI) which shows the difference in absence between Mondays and Fridays. She asserts that if the absence were due to illness only there would be no reason to expect any difference in absence between different days of the week. A high BMI argues, according to BEHREND for high voluntary absenteeism which, in turn might be a measure of the sense of responsibility of the individual The desire to work varies and is said to be lowest the day after a holiday The conscientious worker goes to work even if he does not feel like it, while the less conscientious worker remains at home GORDON et al (1959) feel, however that the BMI should not be used in the evaluation of the sense of responsibility of the workers He stresses that absence, verified by a medical certificate reaches a higher peak on Mondays than does short absence because of sickness He is of the opinion that if a person does not feel really well at the end of the week, he will, if he is conscientious, go to work as usual in the hope that he will recover during the Sunday If he has not recovered, he must perhaps remain at home on Monday and by then the disease might have progressed with the result that he will be away from longer than would have been necessary if he had not gone to work on the Friday GORDON et al. are of the opinion that the BMI might very well be a sign of a keen sense of duty LINDGREN who in 1957 studied a group which had been punished by disciplinary measures found the duration of absence to be longer than in other groups but he found no difference in the distribution of absence among the days of the week

The BMI is calculated in different ways According to BEHREND one should base the BMI on the total number of individuals absent on the various week-days HENRIKSSON studied the number of one-day absences while *GORDON et al.* studied the day of the week on which the absences started LINDGREN compared absence on the day

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SIZE OF COMPANY

Behrend (1951) and Elffvengren (1953) reported a clear tendency absence to be higher in larger companies. Henriksson (1954) however found no significant difference in his series f employees. Neither did he find the size of offices or workshops to have any significant influence on absence. F r females working in groups f more than 20 absence was, however higher

REQUIREMENTS OF WORK

Regarding educational requirements see p 1

Some jobs require peopl with a high sense of responsibility others with a high degree f practical experience. Pugh et al. (1939) found that salaried employees in responsible positions had a low sick absence In their investigation it was possible to separate responsibility from educational level, which is therwise not easy

All investigations indicate th t occupation placing high demands on phy ique show a high frequency f absence (Behrend 1951 Elffvengren 1953, Liddell 1954, For man 1958, Shephard et al. 1957)

The rôle played by psychical demands regarding ab erlined

1

demands placed upon a person are more than he can cope with. LINE (1951) stressed that absence is lower among persons working together with others than among those who are more or less isolated. BROCH (1959) was of the opinion that high frequency among crane drivers is due, among other things to the isolation combined with the relatively great risks of damaging material and injuring fellow workmen. Two thirds of all crane drivers complained of mental stress

Places of work vary regarding environments climate, noisiness, temperature, illumination, contamination of the air etc. NORMAN et al. (1956) stressed, however that people working in a given factory office or occupation represent a natural selection. Only those who believe they are able to manage the work remain at the job and those who realize that they are unfit for it finish and seek employment elsewhere However NORO (1949) and GORDON et al (1959) found that people with open air occupations were away from work less than indoor workers. The seasonal variation of absence of the former group was also less LIDDELL (1954) found a higher sickness absence among surface workers in mines than among those working below which might, however be due to the larger number of partially disabled persons among the former But he found a higher voluntary absenteeism among face workers who have the heaviest and most risky mine work.

A search of the literature failed to reveal any comparison between full time and part time workers HENRIKSSON (1954) excluded, for example, part time workers in his analysis BEHREND (1951) reported only that the absence had decreased since the introduction of the 5-day week. As for shift work, investigations have also been made in Scandinavia (THIIS-EVENSEN 1957) which showed that absence was lower among shift workers, which was believed to be ascribable to selection

CONDITIONS OF EMPLOYMENT

It is clear from what was said above that absence varies with the requirements placed on the workers It is therefore reasonable to assume that a difference should be expected between salaried employees and workers BERGMAN (1949) who analyzed a series in the foodstuffs industry found a statistically significant overmorbidity among factory personnel regarding diseases of the digestive tract, skin diseases and acute infections and, in particular joint and muscle diseases On the other hand, office clerks had a higher frequency of absence because of respiratory tract infections gynaecological diseases and general weakness The Statistical Office of Stockholm (1950) found a somewhat lower absence for salaried employees STRÖM (1957) reported that female workers were away from work twice as much as salaried female employees. In most of the cases the investigations were carried out either on workers or on salaried employees only so that the series are not comparable

BASIS OF CALCULATION AND LEVEL OF INCOME

At least in Sweden salaried employees are paid by the month and workers by the hour or by the piece Comparison between monthly salary and hour money must therefore be considered in the discussion of conditions of employment Stockholm s Statistical Office (1950) found no fundamental differences in absence between those who were on piece work and those who were on time work.

As to the level of income data available in the literature are contradictory NORO (1949) and SHEPHARD et al. (1959) found a higher frequency rate among those with a higher income EKENDAHL

et al. (1950) found absence to increase with alary among unm rried men. Married men, on the other hand, howed no such tendency and mong the w men bsence decreased with increasing salary. FRIBERG et al. (1953) were unable t find any correlation between frequency of illness and income. Health Statistics (1958) found absence to vary inversely with the f mily income: Low income—high frequency. This fact r l urely related t educati nal level, requirement of the j b, condition of employ ment, and the factors

STATE OF LABOUR MARKET

In th discus ion f w rking cond tions the tat f the labour market hould perhaps also be considered. Many u thors, including BEHREND (19 1), claim that bsenteeism has become much greater problem since th second w rld w and the subsequent scarcity of labour. ELFVENGREN (19 1) expressed the iew that the distinct difference between absenteei m in Sweden nd in Finland is d mainly to diff rences in vailability of labour.

HENRIKSSON (19 1) however com pared absence during years when labour was scarce and ears when labou w les scarce and found no difference in bsence. LYNCH (1943) investigated the ca se f high bsence d ring scarcity f labour d ring the wa in the USA. He found, above all, that the mortality increased and concluded that t w reasonabl to suppose that the increase in m rtality wa accom panied by an increase in m rbidity. Secondly he poi ted out that ind try must employ les experienced person and a higher percent ge f f males duri g wa tim. Thirdly the a erage age f the w rkers is m ch highe

ABSENCE DUE TO MEASURES TAKEN BY EMPLOYER

Travell ng and work away from factory. If a person is sent t work at some place where it is not possible t regist r the a tual n mber of hours h i w rking, g., bu iness trips, sembling ma chines or the l k away from hom the absence appearing on hi work card is generally classified as b sence on bu iness. It is thu n t a quest on f bsence from w rk, but registration is important, ince nothing is known of the state of health of the person d ring s ch absence.

Temporary dismissal. In the vent f insufficient orders or stoppage of work because f break-down of machinery or the like it may be neces ry to giv notice to some of th workers

EFFECTS OF CONFLICT OR AGREEMENT BETWEEN EMPLOYERS AND EMPLOYEES ON ABSENCE

A conflict may result i a trike or lockout. The ignificance f these fact rs ries considerably from one country t another. BEHREND (19 1) f r example tated that volunt ry absentee m in England i just as important a trikes.

According t S edi h law working ondition are decided b the parties concerned. In principle trikes are forbidden as long an agreement is in f rce. It is also generally greed that in the event of contemplated trike or lockout the trad union or the employers hould be gi en a tice in good time in rder t provide pos ibility of coming t some greement without

ary

a strike or lockout Apart from these exceptions, workers have the right to strike and employers have the right to announce a lockout. Civil servants have not the right to strike, but they can exert pressure on the state by giving notice and blockading the vacancies thus arising Thanks particularly to the Saltsjöbaden agreement, strikes or lockouts are now rare. In some places, however short local wild strikes still occur These may be due to some local trouble and seldom have any influence on the population in general (MICHANEK 1958)

The working times described by law can be modified by agreement between workers and employers. Thus, the law prescribing a 45 hour week is applied in such a way that during the greater part of the year the workers put in longer hours in order to have days off e g., Saturdays during the summer months The examples given concerning factors due to measures taken by the public, by workers or employers have one thing in common, namely that they can influence sick absence statistics, since they shorten the total duration of time a person may be absent because of sickness FORTUIN (1955) stressed for example, on comparison between a company in the USA and a similar company in Holland, absence was found to be greater in the American firm during the last world war The difference was due to the fact that in the USA the young and healthy individuals were called up for military services which was not the case in Holland

For calculating the number of whole-year workers for the total period on which sick absence can be based, one may use the following formula given by STRÖM (1957)

$$X = \frac{\Sigma A - [(F^{I} + F^{II} + F^{III} + \ldots)]}{B}$$

where A is the number of work days offered to those who have been in the employ of the company for one year F^{I} F^{II} etc., the number of days of absence because of cause I II etc., and B the number of ordinary workdays per year

HENRIKSSON (1954) questions whether it is justified to take individuals who, because of any of these factors have had a much shorter exposure time than the others together with those who could be observed by the company for the entire observation period

EFFECT OF PUBLIC MEASURES ON ABSENCE

The working hours are regulated in different ways by law

Holidays The number of holidays a man is entitled to in Sweden was not regulated by law until 1938 Already before that time however most employees and three fourths of workers belonging to trade unions had holidays For trade union workers it was, however only 5 days a year but in 1938 it was increased to 12 days and in 1958 to 18 days The holidays should with certain exceptions be taken at a single stretch (MICHANEK 1958)

Overtime Overtime may be regarded as negative absence and it is due to the fact that the ordinary working hours are prescribed by law There is no doubt that the demand for a reasonable number of working hours during the 19th century was well founded from a medical point of view An 8 hour day was demanded at an international congress in Paris in 1899 and in Sweden this became law in 1919 In recent years claims have been made for a further shortening of working hours but this is no longer of any importance from a medical point of view (MICHANEK 1958)

The law of 1957 regulating working hours in Sweden which is still in

force, prescribed that the number of hours per week in 1958 should not exceed 47 in 1959 46, and in 1960 45, or t most 9 hours a day

All time exceeding that laid down in the law was to be regarded as overtime

According to the laws for the protection of workers, overtime should not exceed 48 hours per 4 week period, or 200 hours per year In addition, so-called emergency work is allowed but only for 7 hours a week by workers below 18 years Further overtime can be granted, but at most 150 hours a week.

It is widely claimed (Gavayen 1943, Behrend 1951) that sick absence is influenced by the number of working hours and, particularly by overtime. The actual significance of overtime has however, not been investigated. With the present laws for the protection of workers, overexertion or fatigue of healthy workers may be neglected except, of course among those doing extra work in the evenings or the week ends (Michanek 1958).

Military service, public commission, imprisonment, appearance at court of law etc., are other examples of absence due to public measures

ABSENCE WITH PERMISSION

Periods lost because of absence with permission are on the average much lower per person per year than absence because of disease In Forssman's (1958) series it was about one fourth of sick absence On close analysis of lea e of absence It will be found that those who ha to take care of children or old persons require more lea e of absence than others (Elfvengren 1954) She also found leave of absence to be high in rural districts which might be due to people requiring time to look after small farms, etc. Elfvengren also pointed out that while sickness causes absence of days or weeks, absence with permission is usually granted for hours or part of a day

ABSENCE AND SOME INDIVIDUAL BASIC FACTORS

SEX

It is well known that the sexes differ regarding absence from work. It is generally tated that women are absent twice as much as men (Ekendahl et al. 1950 Noro 1919 Frisberg et al. 1953 Henriksson 1954 Forssman 1958).

Lynch (1913) found women t be absent more than men during th entire period of his tudy i.e., 1913—191 Elfvengren (1953), how ver found hardly any difference between the sexes in Finland, and the unmarried women were if anything, away from work le than the men

In an investigation in 1906 of the General Post Office in Sweden, the "disability rate" was equally high among post men and post women, but the "frequency rate" was twice as high for the women. This can be partly explained by the differences in the age distribution of the two groups. Below the age of 40, the women had a higher "disability rate" than the men, while above 40 the men had a higher "disability rate" than the women.

It is ob ious that many women have in reality two occupations, i.e., their occupation as such and the care of their home and the children or of other relatives. Ström (1957) Elfvengren (1933) Ekendahl et al. (1950) Henriksson (1954) and Behrend (1958)

a strike or lockout. Apart from these exceptions, workers have the right to strike and employers have the right to announce a lockout. Civil servants have not the right to strike, but they can exert pressure on the state by giving notice and blockading the vacancies thus arising. Thanks particularly to the Saltsjöbaden agreement, *strikes or lockouts are now rare*. In some places, however, short local wild strikes still occur. These may be due to some local trouble and seldom have any influence on the population in general (MICHANEK 1958).

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Overtime. Overtime may be regarded as negative absence and it is due to the fact that the ordinary working hours are prescribed by law. There is no doubt that the demand for a *reasonable number of* working hours during the 19th century was well *founded* from a medical point of view. An 8-hour day was demanded at an international congress in Paris in 1899, and in Sweden this became law in 1919. In recent years claims have been made for a further shortening of working hours, but this is no longer of any importance from a medical point *of view* (MICHANEK 1958).

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LEVEL OF EDUCATION

Most thors have fou d absence from work t ary inversel w th the level of general education BEHREND (1958) reported a lowe frequency among killed persons tha mong nskilled persons. HENRIK SON (1951) found the absence mong males t be in ersely proportional to the level of education This tendency w however not demonstrable among the female employees

DURATION OF EMPLOYMENT IN THE SAME COMPANY

The res lt f n comparison between absence of employees who hav been in the service f the company for a long t me and hort tim respecti ly will, of course d pend on definition of short and long In some nvestigations the thors ha e compared those who started and finished a j b within one year and those wh had worked the whole year r more. BEHREND (1958) for example, found voluntary absence to be higher among newly employed persons but stressed that it was difficult to eliminate the influence f ge in the calculations Those wh had finished their work within ne year had higher frequency of absence than those who had been in the company f r more than one year (HENRIKSSON 1954) One can also compare those who often change their jobs (shifters) with a more stable group EKENDAHL et al. (1950) found ick absence to be lower for unmarried men and women among the shifters but higher for married men and women than the stable group HENRIKSSON (1954) found support fo the theory that hifters have a higher frequency of absentees m. HENRIKSSON warns again t pooling indi iduals who started or finished w rk t the company in one year with those who have been employed the entire year H condemns thi procedure and t tes that absence mu t be corrected for duration of employment. SPRATLING (1956) is of the pinion that the problem can be more or less s tis fact rily eliminated by taking the mean value of the number of employees at the beginning and t the end of the year H believes this to be sufficient unless there is a large turno er of personnel. The error will increase w th the observation period Therefore Sick Absence Stati tics ha suggested that this formula be used for each month by itself in the calcula tion of absence of the employees per year

VARIATION OF ABSENCE WITH DIFFERENCES IN RACE, NATIONALITY TOWN AND RURAL POPULATION

CAFAFER (1913) tressed that there re large differences between the absence f whites and blacks H believes however that if comparisons are to be ma de, they hould only be made between imila occupations Then, the differences are insignificant or negligible

ELFVENGREN (1953) compared absence in Sweden and Finland and found much poorer alues for Sweden, which might

stress that women in "unskilled jobs" are absent from work more often than women in professional jobs in clerical work, and, particularly than those in the teaching profession.

In contrast to the *greater sick absence* is the low mortality of females as well as *their ability to tolerate surgical opera*tions for example (Perman 1952) Perman found the duration of life always to be higher for females than males This difference was statistically *significant* in town populations and it was clear for all age groups Boyd gave the differences in morbidity between the sexes (Table 1)

Perman (1952) studied the differences between the sexes in the annual reports of 6 hospitals in Stockholm, and from 11 in other parts of Sweden His material consisted of 16 000 hospital patients Only for 2 diseases namely peptic ulcer and cardiac infarction, was the number of males greater than the number of *females*

AGE

Sick absence varies with age, particularly the disability and severity rate (Gafafer 1943 Forssman 1956 etc.) According to Henriksson (1954) and Kihlberg (1958) however the frequency rate decreases with increasing age Friberg et al. (1953) however found the frequency to increase with age According to the Office of Statistics of *Stockholm (1950) the frequency* of long absences increases with age while that of short absences decreases Ekendahl et al. *(1950) claim that in un*married males the absence increases already at about 50 years while that of *married males shows no distinct tend*ency to increase until about 60 years. *For unmarried females* they found a continuous increase until 60 years, after which it began to decrease The absence of married females was however highest between the ages of 19 and 21 years

Gafafer (1943) found that the frequency of cardiovascular diseases and of cancer increases around *50* years when absence because of mental diseases and rheumatic diseases decreased Norman et al. (1956) reported a careful investigation of absence because of sickness of the personnel of the London Transport Company They found the duration of absence because of illness to increase in duration with increasing age As to the number of spells of absence it showed an increasing tendency for some groups of diseases and a falling tendency for *others*

CIVIL STATUS

Behrend (1958) found voluntary absenteeism to be lower among married men than among unmarried men but the opposite relationship for females Henriksson (1954) reported a certain tendency of the absence to increase for married men but his series was too small

Table 1 *Morbidity among males and females*

Diseases commoner among men	*Diseases commoner among women*
Gastro-intestinal diseases	Biliary tract diseases
Respiratory tract diseases	Thyroid diseases
Heart diseases	Hypertension
(particularly infarction)	Morbus Raynaud
Vascular diseases	Hysteria
Joint diseases	*Chronic nervous fatigue*
Pernicious anaemia	
Leukaemia	

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LEVEL OF EDUCATION

Most authors have found absence from work to vary inversely with the level of general education. BEHREND (1958) reported a lower frequency among skilled persons than among unskilled persons. HENRIKSSON (1954) found the absence among males to be inversely proportional to the level of education. This tendency was however not demonstrable among the female employees.

DURATION OF EMPLOYMENT IN THE SAME COMPANY

The results of any comparison between absence of employees who have been in the service of the company for a long time and short time respectively will, of course depend on definitions of short and long. In some investigations the authors have compared those who started and finished a job within one year and those who had worked the whole year more. BEHREND (1958) for example found voluntary absence to be higher among newly employed persons but stressed that it was difficult to eliminate the influence of age in the calculations. Those who had finished their work within one year had a higher frequency of absence than those who had been in the company for more than one year (HENRIKSSON 1954). One can also compare those who often change their jobs (shifters) with a more stable group. EKENDAHL et al. (1950) found sick absence to be low for unmarried men and women among the shifters, but higher for married men and women than the stable group. HENRIKSSON (1954) found support for the theory that shifters have a higher frequency of absenteeism. HENRIKSSON warns against pooling individuals who started or finished work at the company in one year with those who have been employed the entire year. He condemns this procedure and states that absence must be corrected for duration of employment. SPRATLING (1956) is of the opinion that the problem can be more or less satisfactorily eliminated by taking the mean value of the number of employees at the beginning and at the end of the year. He believes this to be sufficient, unless there is a large turnover of personnel. The error will increase with the observation period. Therefore Sick Absence Statistics have suggested that this formula be used for each month by itself in the calculation of absence of the employees per year.

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Table 2. The average duration of sick absence spells per person under one year (SAS 311) according to some investigations.

Author / Y of publ. / Y of collect. / Type of work	Material (in 1000)	Sex	Mean age	Waiting days	Med. certif. after (day)	SAS 311	Distribution of number of day of sick absence in diagnostic groups expressed in per cent															
							I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XVI	XVII ii	XVII oi
Gafafer (43) 38–41 USA		m		0	7		4.0						9.6	15.0	14.2	4.3		1.8				4.2	3.6
Wada (18) 51–55 USA, Oil company	29	b		0		m 9 f 10							14.1	29.9	17.7				7.9			3.4	6.3
Norman & Spratling (56) 49–52 Engl. Bus drivers	34	m	45	3	3	9.6	2.5	1.6			2.8	5.7	5.7	30.3	18.6			5.5	12.0			3.0	4.4
Norman & Spratling (56) 50–52 Engl. Clerical staff	6	m	41	3	3	8.3	3.4	1.6			4.4	7.4	9.5	34.0	17.5			3.3	7.1			0.7	3.5
Lindgren (57) 55 Sweden, Unskilled labourers outdoors	3	m	46	3	3	28	4.1	0.9	4.1	0.4	7.0	3.3	13.4	15.4	11.6	2.4		1.4	28.3		3.6	1.9	
Netherlands Inst Prev Med 55	79.5	m				15.6	2.5	1.6	0.5	0.2	5.3	5.9	5.3	30.3	14.0	2.7		5.5	13.0	0.1	3.6	8.6	
Netherlands Inst Prev Med 55	10.0	f				16.2	4.6	1.1	0.8	0.9	9.5	3.1	1.3	27.2	12.1	7.2	2.6	2.8	6.2	0.3	4.9	5.3	
Sundell (57) 56 Swed n. Miners	0.7	m	45	3		23	3.4	3.0			9.8	3.4	7.3	8.1	6.4	2.6		3.0	23.5		21.0	4.4	
Sundell (57) 56 Sweden, Forest workers	1	m	42	3		11	5.7	2.5	2.5		2.8	1.2	7.4	7.0	9.8	1.2		2.0	29.0		18.0	12.6	
Forssman et al. Sweden 58, Underground workers							9.2	6.1			3.4		1.9	29.2	11.4	1.8		2.2	10.5		2.3	19.6	
Milan (58) 55 Switzerland Electrical industry	1.9	b		0		9.2		4.6			0.7	4.5	11.5	20.0	11.1				1.7				
Ontario Dep. of Health (59) 57	2.2	m				6.2	2.9	0.7	0.7	0.2	4.3	4.2	5.4	21.8	18.7	2.2		2.0	3.9		4.0	16.4	
	2.1	f				9.3	1.6	2.3	0.7	0.6	2.1	1.7	3.8	15.6	13.9	12.1	3.4	1.1	6.6		7.3	6.3	

m = males f = females b = both sexes I–XVII. See abbreviations. ii = industrial injuries, oi = other injuries.

be due to some extent not only to the scarcity of labour but also to the better sick pay etc.

MALAN (1958) found differences between French Swiss, German Swiss and Italian Swiss workers

FORSSMAN (1959) *reported large differ*ences in statistics for countries with hardly any industries and industrialized countries In some of the former there was a large surplus of labour with the result that, if a work man became ill he could soon be replaced by another Therefore, in those countries there was hardly any absence because of illness In addition, the demand placed on medical care is quite different in non industrialized countries As mentioned previously absence is lower in villages or small towns. One might also imagine that the absence pattern acquired in the country would influence the absence also after migration to town BOS (1958) believes this to explain the improvement of absence in the industry he had studied. According to Health Statistics (1958), however in the USA, no difference was found between the town or rural population. In the rural districts no difference was found between farm workers and others either

A point that should be remembered when comparing members of different nationalities in the same companies is that the latter differ not only in nationality but also by the fact that they have moved from their home country This *century is characterized by mass migra*tion and mass displacement of persons (World Federation for Mental Health Wien 1958) A considerable percentage of the displaced persons are refugees and include those who are mentally ill (SZECSÖDY 1960)

HEALTH STATUS AND ABSENCE

CLASSIFICATION

In many investigations of sick absence, a distinction is made between absence because of accidents during or after work and occupational diseases, on one hand, and other diseases on the other The line between the various groups depends to a large extent on the conditions of the insurance (see GAFAFER 1943)

Sick absence may be classified according to different schemes, some of which are outlined below

Before WHO published its statistical classification of diseases, injuries and causes of deaths various *classification* systems were available GAFAFER (1943) *divided his material into respiratory* diseases digestive diseases and other *diseases.* WADE (1955) extended his classification to include cardiovascular diseases. Classification schemes differing in some respect or another from that of WHO have also been put forward (FORSSMAN 1958, 1961 NORMAN et al. 1956 MALAN 1958) Tables 2 and 3 give the distribution of diagnostic groups reported by various authors. To simplify comparison, the values given have been converted to percentages by the present author In Table 2 it would have been advantageous to have the number of days of absence for the various diagnostic groups, but this information was not always available Table 3 gives the number of spells of absence and Table 2 the number *of days of absence* The tables give the year of the investigation, the country of the investigation, sometimes a branch of the industry or the company studied, sex, number of spells and days of absence per individual, period of absence permissible without production of medical certificate and the number of waiting days before the spells *of absence were included in the* statistical calculation *Despite the hetero-*

T ble 2. The average duration of sick absence spells per person under one year (SAS 311) according to some investigations.

Author Y of publ. Y of collect. Type of ork	Material (in 1000)	Sex	Mean age	Waiting days	Med. certif. after (da)	SAS 311	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XVI	XVII ii	XVII ol
							Distribution of number of days of sick absence in diagnostic groups expressed in per cent																
Gafafer (43) 38—41 USA		m		0	7		4.0						9.6	35.0	14.3	4.3		1.8				4.2	5.4
Wade (55) 51—55 USA OB company	29	b		0		m 9 f 10							14.1	29.9	17.7				7.9			3.4	6.3
Norman & Spratling (56) 49—52 Engl. Bus drivers	56	m	45	3	3	9.6	2.5	1.4			2.8	5.7	5.7	30.3	18.6			3.5	12.8			3.0	6.4
Norman & Spratling (56) 50—52 Engl. Clerical staff	6	m	44	3	3	8.3	3.4	1.6			4.4	7.4	9.5	34.0	17.5			3.3	7.1			0.7	3.5
Lindgren (57) 55 Sweden, Unskilled labourers outdoors	3	m	46	3	3	28	4.1	0.9	4.1	0.4	7.0	5.3	13.4	18.4	11.6	2.4		1.6	28.3		2.6	1.9	
Netherlands Inst Prev Med 55	79.5	m				15.6	2.5	1.6	0.3	0.3	8.3	5.9	5.3	30.3	16.0	2.7		1.3	13.0	0.1	2.6	8.6	
Netherlands Inst Prev Med 55	18.0	f				16.3	4.6	1.1	0.8	0.9	9.5	3.1	1.3	37.3	12.1	7.2	2.6	2.8	6.3	0.3	4.9	5.3	
Sundell (57) 56 Sweden, Miners	0.7	m	43	3		23	5.6	3.0			8.8	3.4	7.3	8.1	6.4	2.6		1.0	21.8		21.0	6.4	
Sundell (57) 54 Sweden, Forest workers	1	m	43	3		11	3.7	2.5	2.5		2.8	1.2	7.4	7.0	9.0	1.2		2.0	29.0		15.0	12.6	
Forssman et al. Sweden 58, Underground workers							9.2	4.1			5.4		1.9	29.2	11.6	1.8		2.2	10.5		2.5	19.6	
Malan (58) 55 Switzerland Electrical industry	1.9	b		0		9.2		4.6			8.7	4.5	11.5	20.0	11.1				1.7				
Ont rio Dep. of Health (59) 57	2.2	m				5.2	2.9	0.7	0.7	0.2	6.3	4.2	5.6	31.8	18.7	3.3		2.0	3.0		4.0	16.4	
	2.1	f				9.3	1.6	2.3	0.7	0.6	2.1	1.7	3.3	15.6	13.9	12.1	2.6	1.1	6.6		7.3	6.3	

m = males f = females b = both sexes I—XVII. See abbreviations. ii = industrial injuries. ol = other injuries.

Table 3 *The period prevalence rate (SAS 310) according to some investigations.*

Author Yr of publ. Yr of collect Type of work	Material (in 1000)	Sex	Mean age	Waiting days	Med. certif. after (days)	SAS 310	Distribution of the period prevalence rate among diagnostic groups expressed in per cent I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XVI	XVII a	XVII d
Gaf fer (43) 38—41 USA		m		0	7		1 1						2.1	59 0	17 1	1.2		1.5				2.1	4 7
Gaf fer (43) 38—41 USA		f		0	7		0 5						1 3	51 0	17	8 0		1 2				0.3	4 1
Natvig (55) Norway 49 Workers		m					10.2	1 0	0 1	0.2	5.5		1 8	18.7	10 1	2.4		2 2	18.?		8.0		17 7
		f					6.2	0 9	0.2	2.6	8.0		3 5	17 4	7 1	6 4	2.8	3.2	23.0		10 4		8 2
Natvig (55) Norway 49 Salaried employees		m					10 5	1 3	0.2	0 1	9 0		6.7	24 6	9 7	3.3		1.2	17 0		4 4		12.1
		f					5 7	1 3	0 6	1 0	10 5		3 6	18 7	5 9	7 2	1 5	1 5	31 4		4 5		6 6
Ward (55) 51—5 USA Oil company	28	b		0		m 1 1 f 2.8							1 6	47 7	26 0				5			1.3	3.3
ܝ therlands Inst Pre Med 55	79 5	m				1 1	0 7	0 2	0.3	0 1	4.5	3.0	1 6	14.5	15.3	1 5		4 9	8.8		0		7 6
	100 0	f				1.8	0 8	0.3	0 4	0 6	8.5	1 7	0.8	14 3	12.8	9.5	0 3	..1	3.3		11 3		3 3
Norman & Spratling (56) 19—52 Engl. Bus drivers	56	m	45	3	3	0.3	1 4	0.7			2.1	2.3	3 0	40 0	15 1			5 4	11 0		5 0		0
Norman & Spratling (56) 19—52 Engl Salar empl.	6	m	44	3	3	0 4	1 9	0 7			2.4	2.3	1 2	19 0	12.6			1 2	7 5		0 1		3.2
	1 6	uf	30	3	3	0.5	3.3	0 3			4 4	2.0	2.1	18.1	10 6	1 2		5.5	4 0		0 6		3.9
	1 9	mf	37	3	3	0 7	2.0	0 6			3 9	2.6	1 7	15 0	10 6	3.5		4 7	7 5		0 4		3 0
Strøm (57) 4 55 Norway Food staff	0 9	f	36	3	3	2.3	1 5	0 7	0 6	1 3	6 5	1 8	0.5	30 0	7 7	6 7	3 7	2.3	20 0		11 0		9.3
Forssman et al 58 Sweden Underground factories							0 6	0 3			1 7			10 1	20.3	0 6		2 3	7 1		3 2		20 0
Health Statistics USA 58		b		0		3.5 1 1	12 0							58.5	3				13 4				
Ontario Dep of Health 58 Canada	2.2	m				0 9	1 1	0 2	0 5	0 1	1 1	1 5	1 4	53.2	19 0	1.3		1 9	1 7		8.3		7 3
	2.1	f				2.3	0 5	0 4	0 7	0 1	0 5	1 4	0 7	16.3	19.3	10 8	1 9	0.8	1 7		11 4		3.2
Bishop 59 Canada	5 6	b					0 8	0.3	0 6	0 1	0	1 6	1 0	47 1	20.2	7 7	0 9	1 7	2.8		8.5		5 7
Otterland (60) Sweden Seamen	20						8 7	0 1	1 0	0 1	6 5	16 1	5 5	16.9	27 1	1.3		8.3	0		3.1		1 6

m = males f = females b = both sexes uf = unmarried females mf = married females II = Industrial injuries 1 = other injuries
I—XVII see abbreviations.

geneity of the material, it is striking that all the uthors found a strong predominance of diagnostic group VIII (diseases of the respiratory tract). This applies, above all, to the number of spells of absence per individual. As for disability rate diseases of th respiratory tract were still predominant, but in the investigations carried out in Sweden and Norway diseases f the skeleton and organs of locomotion were predominant. The high frequency of diseases of the respiratory tract is, according to GAFAFER (1943) and ARRENBURG (1959), due, above all, to respiratory tract infections. On comparison between the sexes, the greatest difference wa that in the frequency of accidents. The pattern of the Health Stati tics differs from the remainder This discrepancy can be explained by the fact that the Health Statistics include all ages, while the other investigations cover only working ages.

RELIABILITY OF REPORTED SICKNESS AS REASON FOR ABSENCE

It is often doubted whether all absence reported as absence due to sickness really l due to ill health (BEHRend 1951 1958, SCHÖTZ 1955, PLUMMER 1960) This applies, above all to absence not upported by a medical certificate BEHREND (1951) is of the opinion that particularly short spells of absence include much voluntary absenteeism. In rder t prevent such abuse the employers and insurance companies request a medical certificat which, for practical reasons, is n t alway a ilable t erify such absence It is therefore the rule for mployers and insurance companies to request the production of a medical certificate aly after certain numbe f days, e.g., or sometimes even more In Sweden the G.S.I requests a medical certificat from the 9th day of absence In this connection it might be interesting to note that in a company with 800 employees BOS (1958) found that absence decreased considerably after the com pany had dropped the necessity of the employees producing a medical certificate on the first day of illness

If t is difficult to decide whether absence is due to sickness or whether it is voluntary it is still more difficult to group data according to diagnosis and, as pointed out by NORMAN et al (1956) such classification is only possible for cases in which a medical certificate has been produced But even then certain sources of error are una oidable Sometimes the diagnosis ncludes 2 or more diseases which hould be assigned to different groups of diagnosis In LINDGREN s (1957) series such cases represented about 5 % f the entire m t rial. Moreover it is not certain that the doctor will always gi the most important diagnosis since it is as a rule, enough for him t certify that the patient is ill and that absence from work is justified. This holds in particular for mental disorders, e.g alcoholism, which can prolong sick absence for various ther diseases (LINDGREN 1957)

IATRICAL EFFECT ON SICK ABSENCE

HULMARK (1943) stressed the diff culties the doctors have in judging the working capacity of patient since as a rule, he has only a vague opinion of the working environments of the patient and demand of the work of the latter

NORO (1948) emphasized that many new methods of examination and treat ment often prolong sick absence On the ther hand, FORTUIN (1955) pointed out the risk of waiting too long before a patient is referred for laboratory studies or treatment by a specialist. He also underlines that, although it is part of the doct s training to make a diagnosis rapidly he is not trained t judge the working capacity of his patients. According t FORTUIN doctors do not appear to realize or t consider the economical losses ick

absence implies for the individual and the community

> FORTUIN made an interesting comparison between 9 doctors at the same company with uniform clientele, the same laboratory possibilities the same knowledge of working conditions, etc. He found that 4 of the doctors had given 1000 patients as much as 1 460 work days more sick absence because of respiratory tract diseases than had the "best" four doctors. The difference was found for absence more than 6 days but not in frequency of absence or short time absence

RELATION BETWEEN ABSENCE AND SICK PAY AND CONDITIONS OF HEALTH INSURANCE

GAFAFER (1943) gave a list of 13 factors which can influence comparisons between data from different health insurance companies

1. Is membership of a health company compulsory or voluntary?
2. Age limit for membership
3. Must persons be employed for a certain time before they can become members?
4. Are certain occupations excluded from membership?
5. Do chronic diseases make a person illegible for membership?
6. Resources of organization
7. Effectivity of checking that insurance fees are paid
8. Methods of administration of the insurance company
9. Average wages of members and relationship between sick pay and wages.
10. Personal contact with the patient.
11. Numbers of days of waiting (short spells are often not reported)
12. Retroactive payment also for waiting days if the spell of absence exceeds a certain time?
13. Maximal duration of sick pay?

HUSMARK (1943) *pointed out that it* was important, that sick pay should not be too high *He found that those with* a high sick pay were away from work *because of illness longer than others He* also found support for this assertion in *earlier German literature*

> HUSMARK studied the duration of sick absence because of accidents off duty It was found to vary with the amount of sick pay expressed in Swedish Crowns per day On the other hand, no relationship was found between the duration of sick absence and the normal income of the individual. He found a tendency to overabsence as soon as the sick pay was more than 70—75 % of a person s normal income

NORO (1949) HENRIKSSON (1954) and FAXÉN (1959) arrived at the same conclusion. The committe for the survey of health and medical care in Sweden in 1958 investigated to what extent members claimed support or compensation from the Swedish General Sickness Insurance.

> In Sweden all persons above 16 years are members of the General Sickness Insurance (G.S.I) During sick absence all persons receive a certain basic sick pay They also receive an extra amount depending on their income The total sick pay *i.e.*, basic + extra according to income, is not allowed to exceed 80 % of ordinary income. Compensation is, however limited to at *most 20* Swedish Crowns per day and with no compensation for the first 3 days *of illness. After 6* months illness the rate of compensation *decreases (before 1959* after 3 months illness) and after

2 years illness no more compensation is received from the G.S.I Members are grouped according to 14 classes (0—13) (Bromero et al. 1956)

It was found that those belonging to classes 11—12 took most advantage of the G.S.I. During the observation s period (last half of 1958) 22.9 % of the males and 33 4 % of the females in class 11—12 had received compensation from th G.S.I. as against 9 1 % and 8.4 % in class 0. The age distribution in both classes was the same as the given on page 20 The sick absence was thus highest for males 60—66 years and for females 40—49 years of age

ABSENCE BECAUSE OF PREGNANCY AND CHILD BIRTH

This absence should not be confused with absence because of the diagnostic group "Diseases of pregnancy" According to Swedish law a woman cannot be dismissed because of pregnancy and she has the right to leave of absence in association with parturition. This absence is of relevant interest because it influences the observation period in the same way as the types of absence discussed on p 18. Pregnancy also influences the sick absence pattern by contributing t diseases of pregnancy

ABSENCE FOR NO VALID REASON

Behrend (19 1) focused on this type of absence which she called "voluntary absence" She is of the view that a fair percentage of so-called sick absence is in reality voluntary absence She underlined that "voluntary absence" causes considerable economic losses. In England the time lost by voluntary absence is said to be equal to that due to strikes.

SUMMARY

Chapter _ was concerned with a brief survey of the literature on absence from work. It was found that in the valuation of sick absence the following factors must be taken into ccount: personal situation of worker conditions prevailing at place of work, laws bearing on working hours etc., and health insurance system. Absence for reasons other than sickness must also be considered.

It is clear from compilation of statistics from the literature (Tables 2 and 3) that absence from work is considerable in all countries General agreement on international norms is highly desirable to facilitate comparison between results obtained by different investigators

The data available in the literature is not sufficient t provide answers t the questions posed in the present investigation.

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the predominance of industry in the town has decreased with years. It would appear that the town was in a state of expansion in 19 1 ince as many as 9 % of the inhabitants were employed in the building trade The number of peopl employed in public administration also increased with the years

Table 4 *Distribution of occupations among population of Västerås*

1930		1951	%
Industry	54.6	Industry	50.8
Commerce and		Building	9 0
transport	19.5	Commerce	1 .0
Civil service and		Transport	17.0
free professions	7 7	Civil service	12.0
Agriculture	3.6	Miscellaneous	5.1
Miscellaneous	11.6		

Descriptions of en ironment and condition outs de the fact ry or office sho ld include an account of houses and flats, etc., the subjects are living in. The following account is based on data vailable from the records of th Company and from the fficial tatistics of the town and are probably sufficient to elucidat the general tandard of the home f the employees and workers of the Company

> From the House Office data were obtained on the houses owned or let by the Company and from the Personnel Office data were obtained on those who were living outside of Västerås, and concerning the town in general data were btained from *Västerås stads bostadsräkning* 1956 (tatistics on living accommoda tion).

Owing to the rapid expansion of the Company specially during certain periods it was often difficult t find accommodation for the employees and workers. In order to cope with this difficulty the Company built houses of thei own, agreements were mad with certain builders and th right wa obtained from the Government to dispose of certain houses for the staff of th Company furnished quarters were obtained for the unmarried, special loan were arranged for those who wished to build their wn house, etc

The furnished rooms at the disposal of the Company now total about 1,200 and are situated in hotels of varying class and in some private flats. The cheapest of these hotels were erected after the second W rld War and consist f wooden huts. They contain 150 double-rooms with central heating and accessibility to a pantry wash-room, hower and WC. The rent is 60 Swed. Crowns a month The best furnished rooms re in new houses, double rooms with bathroom, WC and kitchenette in each flat. These doublettes cost 355 Swed. Crowns a month As to the othe flats, it might be mentioned that in 1900 ASEA owned 40 one-room flats and during the first World War a number of flats were built without modern conveniences They thus have no central heating o WC. Of these, some 90 % are flats of one room and a kitchen and the remaining 10 % consist of 2 rooms and a kitchen. Th one-room flats cost 675 Swed. Crowns per year excluding heating.

In 1942, 18 three-room flats and 180 two-room flats and 36 one-room flat were built. In addition, a large number of flats in private houses were subsidized They are all modern with WC, central heating, hower or bathroom, electric or gas stove Since then further flats have been built, and th Company now wn 1,600 flats. The rent for modern flats are given in Table 5

Table 5. *Rent for flats owned by ASEA*

	Swed. Crowns/year	
	lowest	highest
1 room and kitchen	1,421	1,762
2 rooms and kitchen	1,661	2,490
3 rooms and kitchen	2,130	3,696

For employees below 18 years of ge and living awa from home the Company

AUTHOR'S INVESTIGATION

PART I

ABSENCE IN ENTIRE COMPANY

CHAPTER III

ENVIRONMENTS OF EMPLOYEES

THE TOWN

The investigation was carried out at Västerås which is a residential town, situated on lake Mälaren about 120 km west of Stockholm (Fig 29) The town is an old cultural centre that has grown up around the crosspoints of old roads and near a natural harbour

As early as in the 12th century the town was a diocese and in the middle ages it was one of the most important in Sweden and the first gymnasium was founded in Västerås in 1623

For many years the town was small It was not until the last 30 years, or so, that it began to grow and then rapidly Fig 1 The data were obtained from *Statistisk årsbok* 1960

Table 4 gives the distribution of the occupations and professions of the people in Västerås (data obtained from 1st and 2nd ed of *Svensk Uppslagsbok*) Unfortunately the division was not the same in the two years compared, but it is obvious that despite its expansion

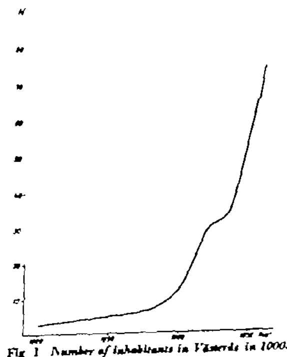


Fig 1 *Number of inhabitants in Västerås in 1000.*

the predominance of industry in the town has decreased with years. It would appear that the town was in a state of expansion in 1911 since as many as 9 % of the inhabitants were employed in the building trade. The number of people employed in public administration also increased with the years.

Table 4. *Distribution of occupations among population of Västerås*

1930	%	1951	%
Industry	54.6	Industry	58.8
Commerce and transport	19.5	Building	9.0
Civil service and free professions	7.7	Commerce	17.0
Agriculture	3.6	Transport	17.0
Miscellaneous	14.6	Civil service	12.0
		Miscellaneous	8.1

Descriptions of environments and conditions outside the factory or office should include an account of houses and flats etc., the subjects are living in. The following account is based on data available from the records of the Company and from the official statistics of the town and are probably sufficient to elucidate the general standard of the home of the employees and workers of the Company.

> From the House Office data were obtained on the houses owned or let by the Company and from the Personnel Office data were obtained on those who were living outside of Västerås, and concerning the town in general data were obtained from *Västerås stads bostadsräkning* 1956 (statistics on living accommodation).

Owing to the rapid expansion of the Company especially during certain periods, it was often difficult to find accommodation for the employees and workers. In order to cope with this difficulty the Company built houses of their own, agreements were made with certain builders, and the right was obtained from the Government to dispose of certain houses for the staff of the Company. Furnished quarters were obtained for the unmarried, special loans were arranged for those who wished to build their own house, etc.

The furnished rooms at the disposal of the Company now total about 1,200 and are situated in hotels of varying class and in some private flats. The cheapest of these hotels were erected after the second World War and consist of wooden huts. They contain 150 double-rooms with central heating and accessibility to pantry, wash-room, shower and WC. The rent is 60 Swed. Crowns a month. The best furnished rooms are in new houses, double rooms with bathroom, WC and kitchenette in each flat. These doublettes cost 355 Swed. Crowns a month. As to the other flats, it might be mentioned that in 1900 ASEA owned 40 one-room flats, and during the first World War a number of flats were built without modern conveniences. They thus have no central heating or WC. Of these, some 90 % are flats of one room and a kitchen and the remaining 10 consist of 2 rooms and a kitchen. The one-room flats cost 675 Swed. Crowns per year excluding heating.

In 1942, 18 three-room flats and 180 two-room flats and 36 one-room flats were built. In addition, a large number of flats in private houses were subsidized. They are all modern with WC, central heating, shower or bathroom, electric or gas stove. Since then further flats have been built and the Company now own 1,600 flats. The rent for modern flats are given in Table 5.

Table 5. *Rent for flats owned by ASEA*

	Swed. Crowns/year	
	lowest	highest
1 room and kitchen	1,621	1,762
rooms and kitchen	1,661	2,690
3 rooms and kitchen	2,130	2,696

For employees below 18 years of age and living away from home, the Company

has erected 22 homes for the boys with 310 beds and one home for the girls with 10 beds These are occupied mostly by industrial apprentices

For those who wished to build their own house the Company formerly gave a loan of 3 000 Swed. Crowns for which they need pay no interest, and which they need not pay back after they had been employed for 10 years This system however was stopped in 1958 Now ASEA instead signs its name as security for a bank loan

The Company has helped some 5,300 of the employees or workers to solve their housing problem The remainder have succeeded in solving the housing problem themselves

Although the data given above may be sufficient to form a rough opinion of the living accommodations of more than half of the employees, nothing is known of how many live in each flat etc. For this purpose data were obtained from the latest town investigation of Västerås in 1956 These data apply to the entire town of Västerås, but since the number of persons employed at ASEA with relatives may be regarded as about 30 000 and thus represent more than one third of the population of the town the housing standard of the town may give an idea of the living accommodation of employees of ASEA It should, however be stressed that those who live in ASEA s bachelors hotels, about 700 are not included in the data given below

The flats are classified according to their quality in Fig 2 They are classified according to availability or non avail ability or central heating WC, bath room It will be seen that the majority of the flats are modern This is only to be expected because the number of inhabitants has increased by more than 100 % since 1936 After 1956 further improvements have been made During 1956—1959 as many as 5 091 flats were built (Table 6) At the same time a

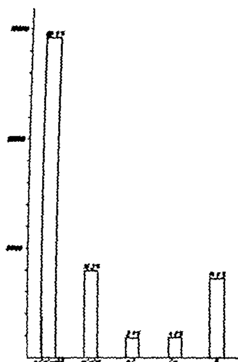


Fig. 2. Distribution of inhabitants in Västerås according to conveniences in flat. WC water closet, CH central heating BR bath-room.

number of old fa hioned houses have been taken down particularly in the centre of the town

Table 6 Number of flats built during 1956—1959

	1956	1957	1958	19 9
1 room	96	291	130	701
1 room + kit hen	30	59	49	11
2 rooms + kit hen	460	418	225	358
3 rooms + kitchen	49	433	334	438
4 rooms + kitchen	148	187	225	1 0
>4 rooms + kit hen	59	60	110	93

The number of persons living in each type of flat is given in Fig 3 The number of inhabitants per flat is al o given

According to the National Hou ing Board the flats are generally well adapted to the size of the families The number of large flats occupied by very small families is small, barely 200 with one or two persons in flats of 5 room and a kitchen and about 200 flats with

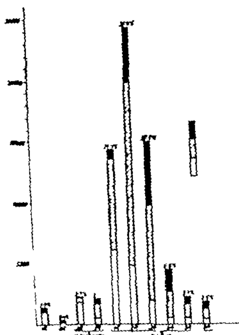


Fig. 1. Distribution of inhabitants in Västerås according to size of flat in number of rooms. The number of persons per flat is given in the columns.

at least 5 living in one room and a kitchen. A large number of families of 3 persons or more were however living in flats that were too mall, *i.e.*, more than 2 *persons in one room (kitchen not counted* as room) According to the norm of 2 persons in each room, 3,663 households with 14 900 members were crowded This implies that 16 % of all f milies and 28 % of all f milies of more than 3 members had flats that were too mall for them.

This crowding of families has continuously increased in recent years. In 1913 the number of members of the family per household was 3.88 and in 1956 it had fallen to 2.87 In 1915, the number of households with 4 members or more was 49 7 % and in 1956, 30 9 %. The town investigation of Västerås also includes comparisons with 7 other towns, of which 4 were of roughly the same size, one was much larger and 2 much smaller than Västerås Since the housing situa tion in these towns was studied in a different year the comparison is hardly correct. As to crowded houses, the difference was small (varying between 2.6 and 2.9 persons per household) As to the number of people living under crowded conditions, with more than 3 in the family it varied between 21 and 39 % (Västerås 28 %)

As t the term "crowded" Roos (1949) *says that the minimum room* space to be ideal should be 1.5 persons per room.

The housing situation may also be studied on the basis of data available from the *public housing centres* The ASEA has its own housing office since it proved difficult t employ people with out at the same time being able to arrange for living accomodation. Of those who were employed in 1945, about 400 were on the waiting list for a flat and half of them were classified as "urgent cases" At the end of 1960 the number on the waiting list was 865 f which 200 were still regarded as urgent. Of those now on the waiting list, as many as 73 % wanted 2 rooms and a kitchen, or less 20 % 3 rooms % larger flats.

ENVIRONMENTS OF COMPANY'S EMPLOYEES DURING CHILDHOOD

DAHL, who in 19 4 gave a description of the contacts of Västerås with the rest of the country directed special attention to migration to and from th t wn. H found that in this respect ASEA differed *from other industries.* Those who were employed in griculture forestry and gardening came from families occu-

has erected 22 homes for the boys with 310 beds and one home for the girls with 10 beds These are occupied mostly by industrial apprentices

For those who wished to build their own house the Company formerly gave a loan of 3 000 Swed Crowns for which they need pay no interest, and which they need not pay back after they had been employed for 10 years This system, however was stopped in 1958 Now ASEA instead signs its name as security for a bank loan

The Company has helped some 5,300 of the employees or workers to solve their housing problem The remainder have succeeded in solving the housing problem themselves

Although the data given above may be sufficient to form a rough opinion of the living accommodations of more than half of the employees, nothing is known of how many live in each flat, etc. For this purpose data were obtained from the latest town investigation of Västerås in 1956 These data apply to the entire town of Västerås but since the number of persons employed at ASEA with relatives may be regarded as about 30 000 and thus represent more than one third of the population of the town, the housing standard of the town may give an idea of the living accommodation of employees of ASEA It should however be stressed that those who live in ASEA s bachelors hotels, about 700 are not included in the data given below

The flats are classified according to their quality in Fig 2 They are classified according to availability or non availability or central heating WC, bath room It will be seen that the majority of the flats are modern This is only to be expected because the number of inhabitants has increased by more than 100 % since 1936 After 1956 further improvements have been made During 1956—1959 as many as 5 094 flats were built (Table 6) At the same time a number of old fashioned houses have been taken down particularly in the centre of the town

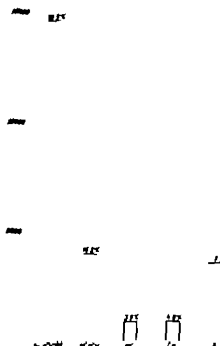


Fig. 2. Distribution of inhabitants in Västerås according to conveniences in flat. WC water closet, CH central heating BR bath-room.

Table 6 *Number of flats built during 1956—1959*

	1956	1957	1958	1959
1 room	96	291	130	401
1 room+kitchen	30	59	49	11
2 rooms+kitchen	460	448	225	358
3 rooms+kitchen	249	433	334	438
4 rooms+kitchen	148	167	245	170
>4 rooms+kitchen	59	60	140	93

The number of persons living in each type of flat is given in Fig 3 The number of inhabitants per flat is also given

According to the National Housing Board the flats are generally well adapted to the size of the families The number of large flats occupied by very small families is small, barely 200 with one or two persons in flats of 5 room and a kitchen and about 200 flats with

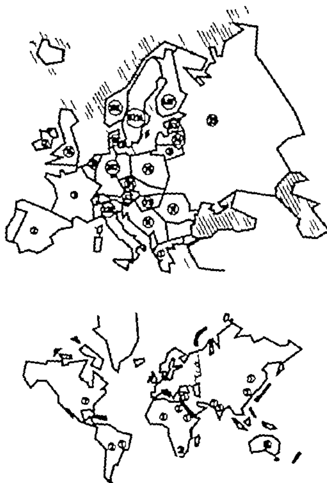


Fig. 5. *Distribution of all employees according to place of birth.*

Table 7 *ASEA servants born abroad*

Employees	men	573
	women	177
Workers	men	774
	women	238
Total		1761

Fig 4 *Distribution (%), according to place of birth of employees born in Sweden.*

pied in the retail trade, those employed in small industries from a somewhat larger branch including the industrial towns in the middle of Sweden, shop-assistants clerks etc from the large towns, while the workers in ASEA were recruited from the whole of Sweden mainly from small towns

As mentioned DAHL studied both migration to and from the town, but only in Sweden In an attempt to form an opinion of the environments of the service of the Company during childhood there towns of birth were charted It would have perhaps been more correct to study where they had spent their childhood rather then their place of birth, but only the places of birth are registered in the record office

The distribution of places of birth are given in Figs 4 and 5 In the map of Sweden the distribution is given in per cent for each county

It is obvious from the map that the town population was never enough to satisfy the requirements of ASEA who had to recruit many of their servants from outside the town In the beginning it was however mainly from the town and the immediate surroundings Gradually however the recruiting area was extended, and during the 30ies people were employed from all over the Sweden and then a relatively large number from those counties with unemployment particularly in the north After the second world war it was necessary to import employees from abroad Many of these foreigners had come to Sweden as refugees others had come voluntarily because conditions here were better for them then in their home countries and some were recruited directly abroad, *e g.*, in Italy in 1947

In Fig 4 the places of birth are distributed among counties If instead we divide the population into two groups according to their places of birth, towns and the rest of the country it will be seen that 45 % of the salaried male employees and 35 % of the male workers were born in towns This may be explained by the fact that the possibilities of higher education have so far been much better in the town than in the country

The number of the Company's servant born abroad are given in Table nr The Table refers to the year 19 9

Places listed as towns in *Statistisk Årsbok* 1914



Fig. 5. *Distribution of all employees according to place of birth.*

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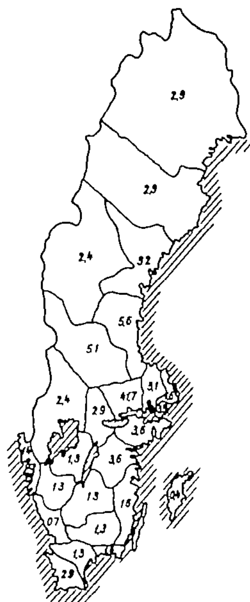


Fig 4 *Distribution (%), according to place of birth, f employees born n Sweden.*

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The number of the Company s servants born abroad are given in Table nr 7 The Table refers to the year 1959

Places li ted t wns I *Statistisk Årsbok* 1914

riages, tomic power stations, cranes, lifts, rolling staircases, motors, apparatuses, power and illu mination installations.

The Office of the Central ASEA is situated in Västerås In the factories in Västerås generators, motors, low voltage apparatuses, ovens, cranes are manufactured.

The salaried employees work in premises erected in 3 different periods, the last accomodating 1,200 salaried employees, was taken into use in 1960 The lack of floor space in 1959 was troublesome, and it was necessary to hire premises in some 20 houses in the town Some of the salaried employees are w rking in laborat ries close t the work shops, others in laboratories elsewhere. At present, existing laboratories are being extended, and new ones erected.

The positions of the main premises of the Company are given in Fig 8. It might be mentioned that the officies satisfy the highest hygienic requirements.

The wo kers are divided among 6



Fig. 7 Number of persons employed at ASEA 1915–1960

fact ry districts (Fig 8) The workshops re also being expanded, and old fact ries are being modernized Most of the work shops are modern and, with but few exceptions, sati fy all hygienic requirements that can be placed on a modern industry

In 1929 a docto was employed for treatment of accidents. In 1946 a Medical Department was started for all medical care and another for the care of accidents. Since 1930 all applicants must undergo a medical examination before they are employed

At present 3 full-time doctors are employed. In principle half of the time f doctors is to be spent on care of the sick and the other half on health service, which includes pre-employment examinations, periodic control of certain categories of workers, tracing of risks for health, health education, etc.

DISTANCE BETWEEN HOME AND WORK

A person s house is of importance not only regarding its size and standard but also regarding the distance and the time from home to work. Fig. 8 hows the main premises of the Company and the number of workers employed at each factory The concentric rings with the centre in one f the factory district are based upon the travelling time, in minutes by cycle, according t Fig 9 The planning office of the Company is at present investigating the necessity of a lunch restaurant on the factory grounds, and therefore it was necessary to tudy the tra elling times. It wa tudied how long it took t cycle from and t work, due regard being paid to the traffic stops, etc. during lunch time Fo the workers, allowance was also made for the time necessary to change clothes so that they required 10 minutes more than the employees (Fig 8, is an example f a series of similar maps in which each factory district is the centre of the concentric rings)

THE COMPANY

The investigation was carried out at that branch of *Allmänna Svenska Elektriska Aktiebolaget* ASEA, situated in Västerås. The Company was founded in 1891 by fusion of 2 companies, the first of which had been founded in 1883. In 1892 the Company moved to Västerås where it now has its main office and largest factories. With the development and extension of the Company and fusion with other companies, The Company has grown into a large group (Fig. 6)

The increase in the personnel of the ASEA Group is given in Fig. 7

The ASEA Group is divided into the Parent Company and Group Companies in which ASEA owns more than 50 % of the shares

Production scheme of Central ASEA the greatest part of the Parent Co Manufacture of electrical equipment for power stations and power nets including generators transformers, apparatuses, rectifiers for all kinds of industry such as steel works mines printing works, paper mills cellulose and textile mills, ships electrical and diesel locomotives, motor and tunnel car

The ASEA Group
33374

Fig. 6 *Survey of ASEA group.*

WORKING HOURS

Salaried employees had a 42.5 hour week in 1959 In order to be able to have the Saturdays off during the summe the number of working hours a day was increased. The f llowing times were applied.

Monday—Friday	7.50—11.30
	13.00—17.00
Saturday	7.50—13 00

15 minutes break for coffee is allowed on Saturdays

Thanks t this lengthening of the working day it is possible for the alaried employees to have 13 Satur days off as well as Friday after Ascension D y Moreover as a kind of rise in wages they were given 2 Saturdays ff during the last quarter of the year

In 1959 the w rkers had a 46 hour week. By working

Monday—Friday	7 00—11.30
	13.00—17 00
Saturday	7.00—12.45

with 15 minutes break for coffee on Saturdays the men were working 48 hours a week. This gave them 13 Saturdays off as well as the Friday after Ascension Day

In some cases working hours were shortened. Among the female workers there was a group of charwomen who worked half a week but did not start until the evenings when work in the factory and offices had stopped.

Among the salaried female employees were many on part time Some of them worked only in the mornings, others in the afternoons. Some of them had Saturdays ff etc. In this way the women were working 50—90 % of ordinary time.

Very few people in ASEA were on shift work in 1959

All workers have 18 work-days holiday a year which is as a rule given in July As f r th salaried employees, the holidays vary according t salary and age between 18 21, 24 or 27 work-days Th holidays must be taken th year after the qualification year but may by special agreement be taken earlier

SALARIES

The salaries f the employees in the "Parent Company" are given in Fig 10 I 1959 the salaries w re n the average a follow

	N mber	Sw Cr./year
Salaried employees		
males	3,500	1 ,5 7
females	1,200	,500

Among the w rkers the mean w ges in V a terås were

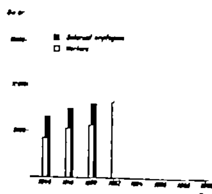


Fig. 10. Mean annual wages and salaries of employees.

Fig. 8. Map of [illegible] showing site of Company's premises. Concentric circles show distances in minutes by cycle during lunch time to and from the factory in the centre. The rectangles give the number of workers of the central factory who have their homes within respective circle sectors (mm — 90 persons).

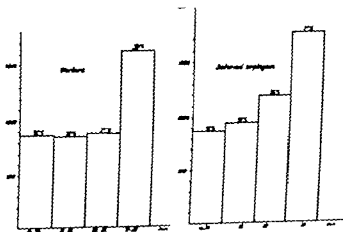


Fig. 9. Distance expressed as time necessary for cycling to and from home during lunch time

WORKING HOURS

Salaried employees had a 42.5 hour week in 1959 In order to be able to have the Saturdays off during the summer the number of working hours a day was increased The following times were applied.

Monday—Friday 7.50—11.30
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SALARIES

The alaries of the employees in the "P rent Company" re gi en in Fig 10 In 1959 the salaries were on the average follow

		Number	Sw Cr./year
Salaried employees			
	males	3,500	1 ,57
	females	1,200	,500

Among th workers the mean wages in Va terås were

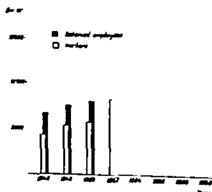


Fig. 10. Mean annual wages and salaries of employees.

	Number	Sw Cr/hr
Male workers		
skilled labour		
workshops	1,100	5 79
foundry	120	6 02
temporary workers		
workshops	1,800	5 11
foundry	280	5 44
Female workers		
workshops	574	3 79
foundry	19	3 98
Male apprentices		
workshops	497	2 98
foundry	14	5.32

*Division of workers into skilled labour*ers and tempo-workers is not otherwise used in this investigation except in the *table above* which was received from the worker's office of the Company Practically speaking it implies that a *skilled labour is* one, who thanks to his training and experience, can solve many problems without the help of a foreman while a tempo-worker need only manage some special manipulations at a certain machine. The borderline between these two groups is very diffuse· much of the work done by tempo-workers requires great skill and the work *of the skilled* labourers has often been rationalized and simplified In summary· Skilled labourers *are doing work that has long been* known as skilled labour tempo-workers have work that has long been called tempo-*work. All skilled labourers* with less than 3 years experience are however classified as tempo-workers

The wages of the workers can be compared with data from *Statistisk årsbok* 1960· average earnings of workers in mining and manufacturing

Sw Cr/hr	*1950*	*1954*	*1958*
adult males	2 73	4.29	5.6[illegible]
adult *females*	1 92	2.96	3 91

HEALTH INSURANCE AND SICK PAY

All employees of the Company are members of the G.S.I ASEA also has a private Sickness and Burial Insurance Fund (ASB) to which all workers belong *automatically except workers without* a clear bill of health on employment, who must be in the Company for 90 days *before they become members* Salaried employees can also become members if they so wish, though relatively few do so

In general salaried employees receive full pay during sickness After a sick absence of 1—6 months depending on age and years a person has been employed, this sick pay is reduced to 50—60 % of his normal salary Sick pay is paid partly by the Company directly and partly by SPP (insurance company) according to a system which need not be discussed *here* In order to avoid over-insurance, the sick pay paid by the Company is reduced by the amount a person receives from the GSI *Allowance* is also made for the fact that sick pay by the GSI is tax free

Among the workers is a small group with a *position* between that of foreman and worker about 200 males who have a weekly wage. They have full pay *during sick absence for 1.5 sick days* number of years in the Company/year The majority of those with a weekly wage *have been* in the service of the Company for many years

Otherwise the workers receive no sick pay from *the Company* They are instead paid by the GSI and ASB The GSI has three waiting days. The ASB pays *one of these* waiting days if the worker has been employed for 2 years 2 days after *4* years and *all the three days after 6 years if the* person is absent for at least 9 days From the 4th day of *illness the ASB pays* 2 *Sw Cr/day*

from 181st day 5 Sw Cr./day and from 721st day (when payment from GSI ceases) 10 Sw Cr /day for 365 days. The total sick pay of the workers will thus be about 80 % of the wages.

In addition, all workers, independently of the time they have been in the Company recei e 100 Sw Cr per 8 week period of sick absence from the so-called help fund

Like all members of the GSI the employees of the ASEA have free medical care in hospital. The costs are paid by the GSI and the county or town. In addition, all members of GSI receive a certain percentage of the fees they have paid to the doctor As far as industrial injuries or other ccidents are concerned, the ASEA also pays that part of the doctor s fee otherwise paid by the employee provided that the medical care is done by the Company s doctor or by a specialist if the patient is referred to him by the Company s doctor The employees can choose their own doctors.

SUMMARY

Data on environments and given in this chapter serve as a background to the sick absence among employees of ASEA. A brief description is given of the town and its growth and of the standard of the houses as well the places of birth of the employees. This is followed by an account of the development of the Company of the industrial branches it covers, and of its factories and work shops The distances the employees have t the factories are also charted. Finally a description is presented of the working hours, wages and sick pay of the em ployees.

Apart from the size of the Company and its rapid xpansion, making it necessary to recruit employees from all parts of Sweden, and the fact that one fifth of the employees were born abroad, the environments of the employees do not differ ubstantially from those in other industries in medium sized Swedish towns

CHAPTER IV

ABSENCE OF EMPLOYEES

SICK ABSENCE IN ASEA

METHOD

The definitions and the nomenclature used are those recommended by the International Conference on Sick Absence Statistics (see page 9)

Concerning the "final day" however we did not follow the recommendation of SAS but included the entire spell of sick absence, even if it exceeded 182 days *The number of days exceeding 182* are given for different diagnostic groups in Table 19

The series was divided according to sex and age, and the salaried employees and workers were treated separately Since the absence of the salaried employees was treated statistically in a somewhat different way than that of the workers, the absences of these two groups are not directly comparable

As far as the salaried employees are concerned the absence was calculated in days with an accuracy of half a day and holidays were included. The number of spells and the total number of days of absence within the year of each employee are noted separately

As for the workers, absence was registered in hours with an accuracy of 0 1 hour Holidays were not included. Only the total number of hours of absence within the year of each individual was noted thus not the number of spells

Absence was studied for the year 1959 Since the observation period was one year marked changes occurred among the servants, a point which must be taken into account (see page 18) Instead of following the recommendations on page 18 it was decided to describe each group below by itself

1 Full time employees who had been in the service of the Company throughout the year of observation
2 Part time employees who had been in the service of the Company throughout the year of observation
3 Full time employees who had done military service during the year of observation.
4 Full time employees with absence because of pregnancy during the year of observation
5 Employees who had joined but not left the Company during the year of observation
6 Employees who had been in the service of the Company in the previous year but who left the Company during the year of observation.
7 Employees who joined and left the Company during the year of observation

In the description of these groups it was not possible to take into consideration the period the individual had been in the employ of the Company because no such data are noted in the absence registers The systematic error thereby introduced is a common source of error (though not in statistical publication)

so that it might be worth while con sidering how this error might influence absence statistics. (See also chapter VII.)

In the event of illness, salaried employees should report to the General Sickness Insurance and state the nature of the disease they have. This is necessary if the employees are to receive their salary during absence. For workers it is not necessary to report. A medical certificate must be produced for sick absence of more then 8 days.

Those cases of disease that were reported to the GSI and backed by a medical certificate were allotted to different diagnostic groups according to the classification recommended by WHO. On the basis of this classification, disease curves were plotted, which showed the number of days of absence per 100 employees in the respective groups of diagnoses. Curves were plotted for salaried employees and workers and for females and males.

MATERIAL

All individuals in the employ of ASEA during 1959 and working in Västerås were included in the investigation.

It is clear from the Table that among those wh had worked the entire year the salaried female employees occupied a special position in that their average ge was more than 10 years lower than th t f the remaining groups. Fig. 11 shows that m re than half of the females who had been employed for the entire year were below 3 years of age. It is obvious that the salaried female em ployees are widely used fo subordinate work. This is apparent also from the differences in the salaries between male and f male employees (see page 37).

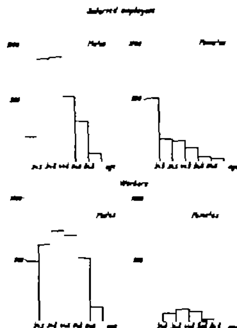


Fig. 11. *Age distribution of employees in service of Company throughout 1959*

Table 8. *Employees classified according to type of work, sex and observation period during 1959*

Employed	Salaried employees				Workers			
	males		females		males		females	
	number	mean age	number	mean age	number	mean age	number	mean age
whole year and full-time	2873	41	973	29	3211	41	301	41
whole year and part-time	5	60	116	38			408	43
whole year minus national service	168	29			160	23		
whole year minus pregnancy			42	23			49	30
joined ASEA in 1939	403	27	196	25	545	25	108	32
left ASEA in 1939	277	56	208	27	371	34	65	40
joined and left ASEA in 1939	157	23	59	27	67	25		

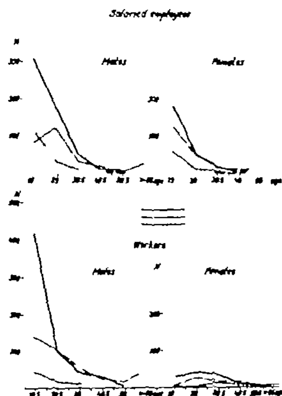


Fig 1.– *Employees at ASEA who have joined ( ), left (b), or both joined and left (c) Company during 1959*

Of the salaried employees on part time work, nearly all were females and they were on the average older than the full time employees. The few part time salaried male employees were so old that it is probable that in this actegory part time was due mainly to sickness As far as the women are concerned, those on part time work probably chose this type of employment because they had so much house-work that they could not manage a full time job

As to military service, the higher average age of the salaried employees suggests that most of them had joined the Company after they have done their first military service and that their absence for military service was largely due to refresher courses etc.

Investigation of the relationship between those who had joined and left the Company within the year of observation revealed interesting differences between the various groups Thus the salaried male employees were engaged at a somewhat higher age than the workers but they remained in the company much longer The difference between the mean age between those who joined and left the Company the same year was 29 years for the salaried male employees as against only 7 years for the workers The difference for the salaried female employees was 2 years and for the female workers 8 years.

Those who joined and left the Company during the observation year were all of low middle age.

The age distribution of the full time salaried employees is given in Fig 11 and of those who joined and left the Company during the same year in Fig 12

RESULTS

The results are given in tabular form in Tables 9—12 for salaried employees and Tabler 13—18 for workers and graphically in Figs 13—19 the curves for absence according to diagnostic groups in Figs 17 18, the effect of age on sick absence in the various diagnostic groups in ıg 19

SICK ABSENCE OF SALARIED EMPLOYEES

Table 9 *Sick absence in days, of salaried male employees in 1959 (SAS 300 and 301)*

Age	Entire year			Joined during year			Left during year			Joined and left during year			MR. service			Part time employees		
	N	300	301	N	300	301	N	300	301	N	300	301	N	300	301	N	300	301
65	65	133	1049	0			24	14	70							1	2	6
55–64	339	316	3078	9	1	3	6	6	294	1	0	0						
45–54	523	831	4530	10	2	4	14	11	92				4	8	51	2	5	127
35–44	811	1255	5006	43	19	64	31	32	194	6	0	0	23	33	115			
25–34	835	1309	4470	146	121	312	122	77	312	32	5	44	81	106	334			
15–24	203	270	899	195	109	315	80	40	122	118	2	11	58	36	170			
Total	2873	4414	19026	403	252	698	277	180	101	157	7	55	160	183	640	3	7	133

Total N 2883
Spells (300) 5013
Days (301) 20656

Table 10. *Sick absence of salaried male employees in 1959 (SAS 310 and 311)*

Age	Entire year		Joined during year		Left during year		Joined and left during year		MR. service		Part-time employees		Total	
	310	311	310	311	310	311	310	311	310	311	310	311	310	311
65	2.1	16.1			0.6	2.9					0.6	2.0	1.6	12.2
55–64	1.5	8.9	0.1	0.3	1.0	49.0							1.5	9.6
45–54	1.6	8.6	0.2	0.4	0.8	6.6			2.0	3.0	2.3	63.8	1.6	8.4
35–44	1.5	5.9	0.4	1.4	1.0	6.3	0	0	1.3	4.6			1.4	5.7
25–34	1.6	3.3	0.8	2.1	0.6	2.5	0.2	1.4	1.3	4.1			1.4	4.5
15–24	1.3	4.4	0.6	1.6	0.5	1.5	0.02	0.1	0.6	2.9			0.7	2.8
Total	1.5	6.6	0.6	1.7	0.7	3.9	0.04	1.3	1.1	3.8	1.4	26.2	1.5	5.3

Table 11 *Sick absence, in days, of salaried female employees (SAS 300 and 301)*

Age	Entire year			Joined during year			Left during year			Joined and left during year			Pregnant			Part-time employees		
	N	300	301	N	300	301	N	300	301	N	300	301	N	300	301	N	300	301
65	4	6	39															
55–64	22	108	786	3	0		8	4	31							2	3	82
45–54	103	211	1126	6	2	15		6	35	2	0					24	40	317
35–44	163	337	2243	15	10	17	17	7	71	3	0					46	73	496
25–34	171	591	2216	46	51	109	52	67	336	10	1	1	10	21	54	31	76	252
15–24	307	1226	3971	129	261	747	124	136	423	34	8	20	32	65	231	12	29	111
Total	972	2679	11397	196	327	887	200	220	896	59	9	21	42	89	285	114	221	1359

Total N 1591
Spells 3542 (SAS 310): 2.2
Days 14815 (SAS 311): 9.3 days

Tab'e 1.. *Sick absence of salaried female employees (SAS 310 and 311)*

Age	Entire year		Joined during year		Left during year		Joined and left during year		Pregnant		Part time employees		Total	
	310	311	310	311	310	311	310	311	310	311	310	311	310	311
≥65	1.5	14 8											1.3	14.8
55—64	4 7	34 0	0 0		0.5	3.9					1.5	41 0	3..	.5.0
45—54	2.0	10 9	0 4	3	0.1	5 0	0				1 7	13.2	1.8	10.6
35—44	3.3	19 6	0 7	1.3	0 4	4 2	0				1.6	10.8	2.6	15.6
25—34	3.5	13.0	1.2	2.5	1.3	6 5	0 10	0.10	.4	5.4	2.4	11 4	.5	9
15—24	2.4	7 8	2.0	5 8	1 1	3.4	0 14	0.4	2.1	7 .	.2	8.6	2.0	6.4
Total	2.8	11 7	1 7	4.5	1.1	4.3	0.15	0 4	2.1	3.2	1 9	11	2.	9 4

SICK ABSENCE OF WORKERS

Table 13 *Sick absence in hours (excl. njuries) of male workers (SAS 301)*

Age	Entire year		Joined during year		Left during year		Joined and left during year		Mil. Service		T t l	
	N	301	N	301	N	301	N	301	N	301	N	301
≥65	125	31871			42	10232					167	4 103
55—64	517	65807	2	0	11	3822					530	7128.
45—54	707	59824	30	2191	21	29*0					758	61935
35—44	737	55905	35	504	57	698	9	15	4	23*	84	63638
5—34	627	39805	85	2334	104	5273	16	113	1	489	853	48014
15—24	498	32651	393	7227	136	8278	4.	369	135	3808	1*04	52333
Total	3 11	285863	545	12256	371	37507	67	482	160	4529	4354	342800

Table 14 *Sick absence in hours (excluding injuries) of m le workers (SAS 311)*

Age	Entire year	Joined during year	Left during year	Joined and left during year	Mil. service	Total
≥65	255		247			.5
55—64	128	0	346			131
45—54	84	73	139			66
35—44	76	14	122		58	6
25—34	64	28	51	7	26	56
15—24	66	18	61	9	28	43
T tal	89	23	100	8	2?	*9

Table 15 *Sick absence (excl. injuries) in hours of female workers (S 45 301)*

Age	Entire year		Joined during year		Left during year		Joined and left during year		Pregnant		Part-time workers		Total	
	N	301	N	301	N	301	N	301	N	301	N	301	N	301
≥65	1				4	1460					4	195	9	1655
55—64	20	3840	1	0	2	68					24	1717	47	5645
45—54	87	15919	8	327	14	4330	6	0			61	7155	176	27722
35—44	110	19708	34	818	15	3467	3	0	3	568	68	9172	233	33733
25—34	77	17350	38	1181	24	3506	4	0	14	5676	48	4037	205	31 63
15—24	6	902	27	653	3	317	1	0	3	250	3	318	43	2433
Total	301	57730	108	2961	62	13143	14	0	20	6494	208	22604	713	102933

Table 16. *Sick absence (excl. injuries) in hours of female workers (S.45 311)*

Age	Entire year	Joined during year	Left during year	Joined and left during year	Pregnant	Part-time workers	Total
65	0		365			49	184
55—64	193	0	34			72	120
45—54	183	41	310			117	158
35—44	180	4	231		190	135	145
25—34	225	31	146		404	84	136
15—24	150	24	104		83	106	87
Total	191	28	212	0	325	108	144

Table 17 *Sick absence in hours, (excl. injuries) of male workers in per cent of time offered*

Age	Entire year	Left during year	Joined during year	Joined and left during year	Mil. service	Total
65	11.4	1.5				13.0
55—64	6.0	32.5	0			6.2
45—54	3.9	11.7	7.8			4.1
35—44	3.3	10.2	2.4	0.3	4.1	3.8
25—34	3.0	4.7	3.9	..9	1.8	3.1
15—24	2.3	5.7	2.9	1.9	..5	..7
Total	4...	9.1	3.4	1.8	2.3	4.3

Table 18 *Sick absence in hours (excl. injuries) of female workers in per cent of time offered*

Age	Entire year	Left during year	Joined during year	Joined and left during year	Pregnant	Part-time workers	Total
65	9	69					
55—64	9.3	3.8	0	0		4.8	19.6
45—54	7.9	43	5.3	0		6.2	7.8
35—44	8.3	76.5	4.7	0	16.3	9.6	9.3
25—34	10.3	22.0	7.3	0	35.0	11.7	9.7
15—24	7.3	11.0	5...	0	6.8	8.4	12.1
Total	8.7	23.3	3.7	0	27.7	9.6	9.4

Table 19 Number of days exceeding "final day" i. e 182nd day distributed according to age diagnostic group and sex

Age Diagn-group	15–24 M	15–24 F	25–34 M	25–34 F	35–44 M	35–44 F	45–54 M	45–54 F	55–64 M	55–64 F	≥65 M	≥65 F	Total M	Total F
I						74								74
II				183									183	
III				114								201		201 114
IV														
V						106	84						84	106
VI							183		150 265		75		333 340	
VII					44		210 183	2	149 366		183 211		54 834	
VIII							17		53				70	
IX							23		10				33	
X									183 183				183 183	
XI						8								8
XII														
XIII			50		7	112	200	60	183	183			6‑3	112 60
XIV														
XVI														
XVII	25		42				193	260	183				443	260

Upper rows = Salaried employees Lower rows = Workers

SICK ABSENCE AND SEX

It is clear from the tables and figures that sick absence was less among males than among females. The average duration of completed or incompleted spells observed per person under observation (SAS 311) *was 1 8 times as great among female as among male salaried employees* (Tables 10 12) and 2 1 times as great among female workers as among male workers (Tables 14 16)

The period prevalence rate (SAS 310) for the female employees was 1 9 times as great as that of the males (Tables 10 12)

SICK ABSENCE AND AGE

SAS 311 shows that as far as males are concerned sick absence increases successively with the age (Fig 14) This is particulary obvious among workers *between 50 and 60 years of age Among* females SAS 311 is *highest in middle age* and then decreases with increasing age (Fig 15) This tendency is particularly marked in certain diagnostic groups (Fig 19). This relation hip is discu sed further on page 50

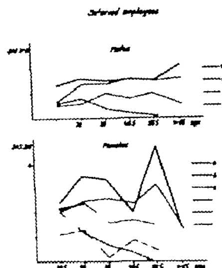


Fig. 13. *Effect of age on period prevalence rate (SAS 310) among salaried employees with different duration of employment 1959 ( ) full time entire year (b) joined, (c) left the Company during year (d) all employees, (e) part time entire year (f) pregnancy during year*

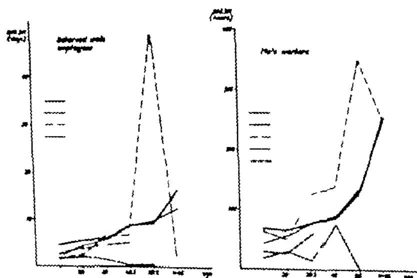


Fig. 14. *Effect of age on average duration of completed or incompleted spells observed per person under observation (SAS 311) among male employees with different duration of employment in 1959 ( ) full time entire year (b) joined, ( ) left the Company ( ) military service during year (d) all employees.*

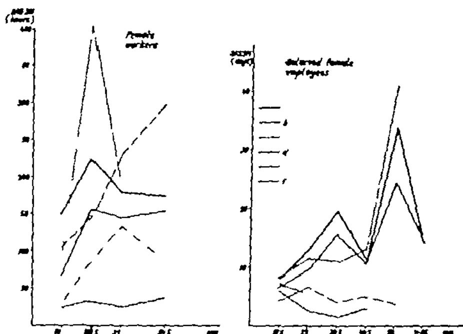


Fig 15 Effect of age on the average duration of completed or incompleted spells absorbed per person under observation (SAS 311) among female employees with different time f employment in 1959 ( ) full time entire year (b) joined, (c) left th company during year (d) all employees, (e) part time during entire year (f) pregnancy during year

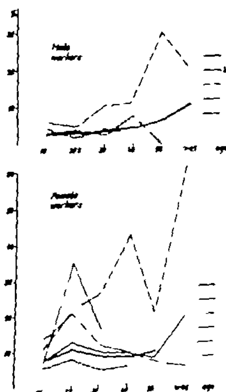


Fig 16. Effect of age on th duration of sick absence excluding nj ries per cent of hours of work offered. ( ) full time entire year (b) joined, ( ) left the company during year (d) all workers, ( ) male military service female: part time entire year (f) pregn ncy during year

SICK ABSENCE OF DIFFERENT CATEGORIES OF EMPLOYEES

As mentioned on page 40 comparison between absence of salaried employees and workers is made difficult by the fact that absence of salaried employees is noted in days including general holidays and absence of w rkers in hours. But supposing the number of working hours during the 365 day of th year to be 145, we shall find a gross absence of the full time workers of 15 1 days for males and 32.5 for females The absence of workers would thus be higher than that of the employees For males the relation hip would be 2.3 1 and f r females .8 : 1

As mentioned on page 41 the part time employees consisted, practically peaking, of females only It i clea from Fig 15 that the part time female w rkers were away from work slightly more than half of that f the full time female workers This may be explained by the fact that in the calculation of sick absence only the part time is reg tered as absence and not the entire d y W th reference t Fig 16 whe the absence is calculated a percentage of work-time ffered, t will be seen that the absen of the part time w rkers was higher except in the highest age classes

Among salaried female employees n the ther hand, the diff rence between the f ll-t me and part-time employees i in ignificant For them the gross number f da of absence is regi tered. Between the ges of 35 and 44 the part tim employees had d tinctly lower bsence expressed SAS 311 A t SAS 310 (period prev lence rat) the absence of the part time mployees wa low r th oughout (F gs 13 15)

Since military service nd pregnancy decreased the duration f period of work offered, the absence found is lower in proportion thereto. Pregnancy however implies n increased ri k of disease which in this material w obvious among female workers, but not among salaried female employees (Fig. 14 15)

Those who joined the Company during the observation year showed a lower absence—if not corrected for shorter observation period—expressed as SAS 310 and 311 in all categories. When absence was expressed in per cent of wo k time offered, no appreciable differ ence was found for the male workers but for the female workers the absence was lower for those who had joined that year (Figs 13—16)

In the higher age classes all except the female empl yees had a higher absence than those who left the Company that year This applies to SAS 311 and was particularly marked when allowance was mad for the working time offered. According to SAS 310 the absence of those who left the Company that year was lower than for the full-time em ployees (Figs 13—15)

CLASSIFICATION OF ABSENCE ACCORDING TO DIAGNOSTIC GROUPS

Absence backed by a medical certificat i.e absence of 9 days duration or more is classified according to the diagnoses in Figs 1 —18, which give the number of days of sick absence per 100 employees

It is clear fr m the figures that the 5 main diagnostic groups of importance in industry are diseases of the respiratory tract (VIII) of the keleton and organs f locom tion (XIII) mental diseases (V) diseases of the digestive tract (IX) and injuries (XVII) Of these group XIII was dominant among the male and female workers and among female alaried employees while group VIII a the la gest among the salaried male employees

In addition, the absence of salaried employees was higher for females in all groups except circulatory diseases (VIII) and dise ses of the kin and subcutis (XII) was the largest among the salaried male employees

4 ann

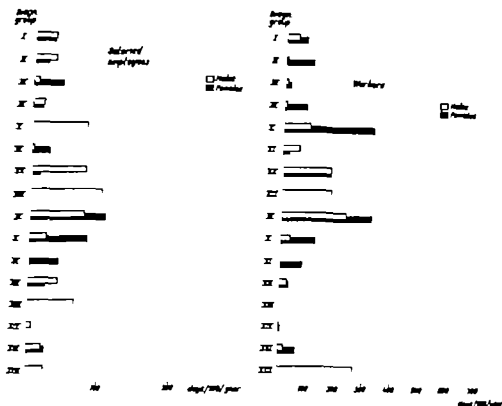


Fig. 17 *Comparison between males and females. Distribution of sick absence within diagnostic groups I–XVII Abscissa number of days per 100 persons per year Note the different scales.*

In addition, the absence of salaried employees was higher for females in all groups except circulatory diseases (VII) and diseases of the skin and subcutis (XII)

Among the workers, the females had a higher absence in all groups of diagnosis except in disorders of the nervous system and of the senses (VI) and cardiovascular diseases (VII)

On comparison between the curves for absence of the male workers and salaried male employees, the absence for the workers was higher in all diagnostic groups except tumours (II) diseases of the skin and subcutis (XII) and un certain diagnoses (XVI)

Among the women it was only allergic diseases, endocrine disorders and meta bolic disorders (III) for which the salaried employees had a higher absence other wise the absence of the workers was much higher

EFFECT OF AGE ON ABSENCE BECAUSE OF DIFFERENT DISEASES

Fig 19 give the effect of age on the absence of diseases in some diagnostic groups

In the males the absence (SAS 311) of cardio-vascular diseases (VII) diseases of the respiratory tract (VIII) increases markedly with age Diseases of the skeleton and of locomotion (XIII) increased rapidly with age among the workers but among the salaried em ployees the effect of age was only moderate As to mental diseases (V) hardly any difference wa found with age among the salaried employees, but a slight increase was found with age among the workers

The shape of the curve for females differed entirely from that for the males Absence was lower for the ages below 25 and above 55 years than in the inter mediate ages Mental diseases (V) and

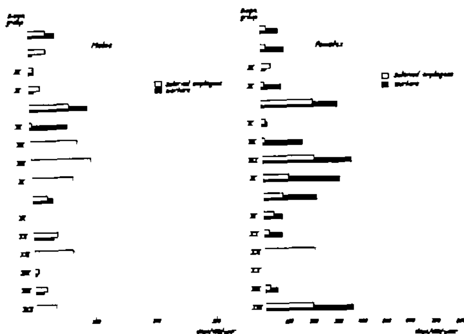


Fig. 18. *Comparison between salaried employees and workers. Distribution of sick absence within diagnostic groups (I+XVII). Abscissa: Number of days per 100 persons per year. Note the different scales.*

diseases of the skeleton and locomotion (XIII) showed a marked peak among salaried employees.

Respiratory tract diseases (VIII) showed a slight tendency to increase with age among salaried employees.

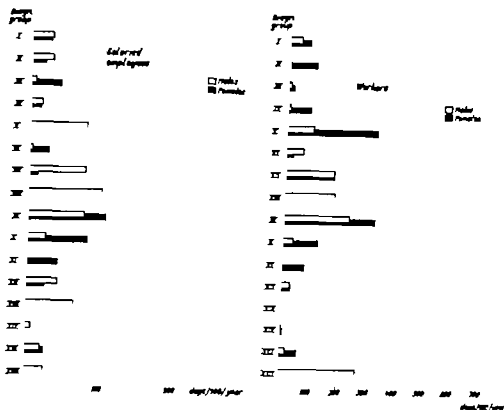


Fig 17 *Comparison between males and females. Distribution of sick absence within diagnostic groups I—XVII Abscissa number of days per 100 persons per year Note the different scales.*

In addition, the absence of salaried employees was higher for females in all groups except circulatory diseases (VII) and diseases of the skin and subcutis (XII)

Among the workers, the females had a higher absence in all groups of diagnosis except in disorders of the nervous system and of the senses (VI) and cardiovascular diseases (VII)

On comparison between the curves for absence of the male workers and salaried male employees, the absence for the workers was higher in all diagnostic groups except tumours (II) diseases of the skin and subcutis (XII) and uncertain diagnoses (XVI)

Among the women it was only allergic diseases, endocrine disorders and metabolic disorders (III) for which the salaried employees had a higher absence, otherwise the absence of the workers was much higher

EFFECT OF AGE ON ABSENCE BECAUSE OF DIFFERENT DISEASES

Fig 19 give the effect of age on the absence of diseases in some diagnostic groups

In the males the absence (SAS 311) of cardio-vascular diseases (VII) diseases of the respiratory tract (VIII) increases markedly with age Diseases of the skeleton and of locomotion (XIII) increased rapidly with age among the workers, but among the salaried employees the effect of age was only moderate As to mental diseases (V), hardly any difference was found with age among the salaried employees but a slight increase was found with age among the workers

The shape of the curve for females differed entirely from that for the males Absence was lower for the ages below 25 and above 55 years than in the intermediate ages Mental diseases (V) and

ABSENCE FOR OTHER REASONS IN ASEA

TEMPORARY DISMISSAL, ABSENCE WITH PERMISSION AND VOLUNTARY ABSENCE

These types of absence are of relevant importance only because they are difficult to distinguish from sick absence None of these absences ffer any economical ad antages, so that there is no reason to assume that the employees or workers con ider such absence desir able. One might, however imagine the opposite. That some sick absence hould by rights be classified as voluntary absence is obvious but in our opinion this is so small that it may be ignored.

Salaried employees may be given time ff if they make up f r the lost time by working longer on some other occasions. In principle then, salaried employees are not given an leav of absence On the other hand, leave f absence is given for personal reasons, .g celebra tion of wedding, 50th anniversary etc At Christma time an employee may be gi en an extra day off if he lives far from the ffice and would not therwise be able t be at home in time for Christma Eve

N record ha been kept in the Company of these types of absence for the salaried employees

As far as workers are concerned, a man may be given time off if he applies for t in advance and it does not interfere with the work in hand. As a rule, it is a question of an hour or so. Voluntary absence is of little importance and here it is mainly a question of lat arrival to work.

The average absence with permission is for female workers 3.3 %, for male w rkers 1.3 %. Average voluntary absence is for female workers 0.0 / and for male workers 0 1 %

OVERTIME

For definiti n f overtime see page 19

The pay f overtime is 150 of normal. This also holds for salaried employees, but not f r head of depart ments

The average overtime is f r male workers 2.6 % and for female workers 1.0 %

The relation between absence with permission, voluntary absence and over time on one hand, and sick absence on the ther is discussed in Part III

SICK ABSENCE IN OTHER COMPANIES IN SWEDEN

A mentioned in the survey of the lit rat re the N tional Social Welfare Board registers absence from work during one week in spring and one week in autumn ea h ea No d t are a ailable on continuous registration of bsence throughout the entire year in different indu tries W therefore collected dat from different companies t btain m terial f comparison with bsence at ASEA in Västerå Thi material included some companies belonging t the ASEA Group nd some outsid companies. It i clear from the T ble 70 that absence wa calculated in different ways so that the data from the different industries are n t strictly comparable Although the nature of the work is given, a thing l known about the requirement placed on the worker g hift work, heaviness of work, de mand on precision etc., and nothing is known f th age distribution among the employees, som times not even the difference between the sexes. The material is a fairly random selection since it was desired t include representatives of large town and of som large and small ind tries The material cannot be regarded a a statistical sample of the

Salaried employees

Females

Males

Workers

Females

Males

Fig. 19 Effect of age on absence in some diagnostic groups. I Mental diseases VII Cardiovascular diseases VIII Diseases of the respiratory tract IX Diseases of the digestive system XIII Diseases of skeleton and organs of locomotion. Ordinate number of days per 100 persons per year

DISCUSSION

WORKING CONDITIONS AND ABSENCE

As in investigations by previous investigators, absence was found to be higher among work rs than among salaried employees in the present material.

Although the classification of a person as a worker or a salaried employee in the present investigation was decided simply by the fact whether he was paid by the week or by the month, broadly speaking worker still has heavier work than a salaried employee In our opinion a given disease will disable worker longer than a salaried employee It is clear from the curves for ick absence that the difference was most pronounced fo diseases affecting locomotion (and, of course, accidents)

When comparing male with female salaried employees it should be remembered that the average age of the females in the present material was 15 years lower than that of the males Nevertheless, the absence wa greater among the females It i obvious that the alaried female employees have on the average more routine work not requiring su h qualifications as the work of the males. Their education is mu h low r well as their respon ibility and their salary According to the literature this in addition to age contributes to the higher absence among females

In the evaluation of the curves f r diseases it will be found, however that over-morbidity is not seen for all diagnoses to the same extent, but particularly for mental diseases, respira t ry tract diseases and diseases of the skeleton and limbs.

Overmorbidity among females is cer tainly due not only to working conditions but lso to conditions in the home. This was confirmed in the present material because it was in those very years when women have most housework that sick absence was highest. Even if this factor be ignored, one might nevertheless naturally expect a higher absence of women Their work is mostly routine work and less interesting, they have no possibility of advancing in the Company and this might possibly result in an increased absence because of mental diseases and sitting at the typewriter all day or at punch card machine might also increase sick absence because of back pain. As to respiratory tract infections it appears only reasonable that the closer contact with children at home hould increase with the frequency of disease in the latter group It is also possible that the young w men are less resistant to infections. One might also imagine that women are more often working in large groups in a single room, which might also increase the risk of infection. But the present investigation gives no information on this point.

On comparison between male and female workers the difference can probably not be explained by environmental conditions during work, since females usually have lighter work than males. Such a comparison, however may not be justified since one hould not compare the absolute requirements of the work,

Table 20 *Sick absence excluding accidents in various branches in 1959 in Sweden*

Branch	Number of inhabitants in town	Salaried employees males number	311	Salaried employees females number	311	Workers males number	311	Workers females number	311
ASEA-group									
Metal	605000	179	9.4[1]d	61	19.0[1]d	330	18.?[1]d	(12	1?.8[1]d)
„	17000	147	6.0[2]d	45	1?.0[2]d	359	104[2]h		
„	6400	281	7.0[2]d	69	17.5[1]d	1372	8.4[1]d	16	10.1[1]d
Agriculture	6800					438	9.8[1]d	(13	1?.?[1]d)
ASEA:s group companies									
Metal	50000	511	9.9[2]d			848	?.6[1]d		
„	15000	482	7.4[1]d	163	12.3[2]d	908	10.?[2]d	(4	11.2[2]d)
Other employers									
Metal	75000	377	9.9[2]d	10	14.6[2]d	1193	14.2[2]d	85	10[2]d
„	75000	1?9	6.5[1]d	69	12.0[2]d	500	80.0[2]h		
„	64500	2312	12.?[2]d	524	1?.9[1]d	2352	96.9[2]h	58	131.9[2]h
„	23000	700	?.2%			2300	3.3%		
Ironworks	21500	1035	10.2[2]d	404	19.6[2]d	4741	13.4[2]d	3?0	18.4[2]d
„	26000	527	?.8[1]d			3530	13.4[2]d		
Mines	25500	60	7.5[2]d	130	14.7[2]d	2460	1?.3[1]h		
Shipbuilding	39 000	1265	70.9[2]h	170	116.1[2]h	4908	158.8[2]h		
Metal	397000	1220	10.6[1]d	464	18.9[1]d				
Leather	222000					210	?%	240	11
Metal	90000	169	1.8%	49	3.9%	917	5.4%		
„	9000	195	7[1]h	131	94[1]h	0	?2[2]h	198	131[2]h
„	4500	70	6.0[1]d	21	10.8[1]d	300	9.9[1]d	5?	15.5 d
Civil service	805000	904	15.?[1]d	361	25.2[1]d	2803	?5.0[1]d	9?	19.0[1]d

[1] Free days included [2] Free days not included d = days h = hours
% (% of working time offered)

Swedish industry The only thing that can be said with some degree of certainty is that absence was higher throughout for the females than for males, and higher for workers than for salaried employees. (See Table 20.)

Uniform registration and collection of data at some central office is obviously desirable

DISCUSSION

WORKING CONDITIONS AND ABSENCE

As in investigations by p evious in estigators, absence was found to be higher among workers than among salaried employees in the present material.

Although the classification of a person as a worker or a salaried employee in the present investigation was decided simply by the fact whether he was paid by the week or by the month, broadly speaking a worker still ha heavier work than a salaried employee In our opinion a given disease will disable a worker longer than a salaried employee. It is clear from the curves for sick absence that the difference was most pronounced for diseases affecting locomotion (and, of course accidents)

When comparing male with female salaried employees it should be remembered that the average age of the females in the present material was 15 years lower than that of the males Nevertheless, the absence was greater among the females. It is obvious that the salaried female employees have on the erage more routine w rk not requiring such qualifications a the work of the males. Their education is much lower a well as their responsibility and their alary According t the literature this in addition to age contributes to the higher bsence among f males

In the evaluation of the *curves for* diseases it will be found, however that over morbidity i not seen for all diagnoses to the same extent, but particularly for mental d seases respiratory tract diseases and diseases of the skeleton and limbs.

Overmorbidity among females is cer tainly due not only to working conditions but also to *conditions in the home* This was confirmed in the present material because it was in those very years when women have most housework that sick absence was highest. Even if this factor be ignored, one might nevertheless naturally expect a higher absence of women. Their work is mostly routine work and less interesting, they hav no possibility of advancing in the Company and this might possibly result in an increased absence because of mental diseases, and itting at the typewriter all day or at a punch card machine might also increase sick absence because of back pain. As to respiratory tract infections, it appears only reasonable that the closer contact with children at *home should increase* with the frequency of disease in the latter group It is also possible that the young women are less resistant to infections. One might also imagine that women are more often w rking in large groups in a single room, which might also increase the risk f infection. But the present investigation gives no information on this point.

On comparison between male and female workers the difference can probably not be explained by environmental *conditions during work*, since females usually have lighter work than males Such a comparison, however may not be justified since one should not compare the absolute requirements of the work,

but instead place them in relation to the capacity of the males or females to cope with them. This was not done so that it cannot be said whether the work placed a greater strain on males than on females

As to working environments the material suggests that poor working conditions and high demands on physical and mental performance increase sick absence

SIZE OF COMPANY AND ABSENCE

As mentioned, ELFVENGREN (1953) and BEHREND (1951) claim that absence is greater in larger companies than in smaller ones HENRIKSSON (1954) however found no correlation between absence and the size of the company The present investigation is based on one of the largest industries in Sweden, and it is quite probable that the individual employee is less inclined to realize his importance to the work of the Company as a whole with the result that he might be more inclined to remain away from work than he would be if he were employed in a small company At the same time he may realize that he belongs to a well founded company which can offer him work even when times are hard This knowledge might have two opposite effects It can give the worker a feeling of safety and less stress a a consequence but it can also result in a certain nonchalance with the result that he might be absent from work longer than is necessary

On comparison between absence in the present material and in other industries (see page 53) and with data from the literature it will be found that absence in this company is not particularly high but if anything low

Since the data given in Table 20 are perhaps not representative of Swedish industries, it is not possible to draw any conclusions from the comparison

ECONOMIC BENEFITS AND ABSENCE

It is widely believed that high sick pay will increase absence from work (HUSMARK 1943 NORO 1949 HENRIKSSON 1954 FAXÉN 1959) The present material did not produce further support for this opinion. On the contrary salaried employees who received full salary during the first month of illness had a lower absence than workers who received 80 % As mentioned above, other factors may also contribute to this difference If we compare the absence of the employees of ASEA with the absence of civil servants, whose sick pay is 80 % of the salary the absence among the ASEA employees will be found to be lower If the ratio between salary and sick pay in this material was of any significance one might imagine it to have the opposite effect. Since it is known that the company pays full salary even during illness the salaried employees try to get back to work as quickly as possible in order not to give the Company the opinion that they are abusing this privilege Another thing is that salaried employees know that the more they are away from their work, the more work will be waiting for them when they get back to their desk.

SICK ABSENCE IN DIFFERENT CATEGORIES OF EMPLOYEES

In the investigation of absence comparison between full time and part time workers is of interest In the present material the part time salaried employees were practically speaking, only females The few males working on

part time because of high age or illness will not be discussed, because it is obvious that their absence must be high.

The female workers consisted mainly of charwomen who worked in the evening after the offices and factories had closed. Most of them could not take work during the day time because they had t take care of their home and children. In the evenings, however they could leave the home and the children in the care of their hu bands That this group have a higher sick bsence (Fig 16) than the full tim employees is perhaps to be expected. In addition, the work f charwomen i relatively hard compared with that f the full time workers.

About 1/10 of the female salaried employees who had been in the service f the company throughout the year under consideration were on part time employment (see Table 11) Most of the part time employees were between the ges of .5 and 45 years, i.e the time when they are needed most t home As mentioned on page 49 the absence was lower for the part time employees It should be bserved that the number of employees, particularly in the higher age-classes, was small, so that no valid conclusion could be drawn. It wa also stressed on page 49 that one day of absence of a part time employee implies less loss to the Company than ne day of absence f f ll-time employee As is apparent from Table 8 th a erage age of the female employees w definitely lower than in the ther groups and also showed the mallest difference in mean age between those who joined and left the company that y

DISTRIBUTION OF ABSENCE AMONG DIAGNOSTIC GROUPS

It hould be realized that the di tribution of absence among the diagnostic groups cannot be more than pproximate. In some cases it is difficult to decide to which group the diagnosis should be assigned. Neither can one be sure that the diagnoses made are really correct or that they say the whole truth. As mentioned in the survey of the literature on page .., the diagnosis is sometimes a false name for some other condition, such as alcoholism, which masquerades under different guises

With these reservations certain tendencies can be discerned. The present analysis gave support t th assumption put f rward by previous workers in this field (Gafafer, Ashenburg etc.) that respiratory tract infections play an important role as a cause of absence in industries, particularly f the number of spells of absence In other words, short absence is most often due to respiratory tract infections. Among the workers in the present material, however it was not respiratory tract infections but affections of the keleton and limbs that were responsible for the majo part f absence It is clear from the Tables 2, 3 that this appears t be the case in Scandinavia but not in other countries This discrepancy is difficult to explain, but the climate in Scandinavia may be partly responsible As mentioned above this cause f absence makes itself felt still more when working condition are poor

In addition t these tw groups ttention mu t be given t mental diseases and diseases of the digestive tract. Particularly mental diseases are according t many pract tioners under-represented in the record on the GSI and thus more common than what is reflected by the profiles of the diagnostic groups.

but instead place them in relation to the capacity of the males or females to cope with them. This was not done, so that it cannot be said whether the work placed a greater strain on males than on females

As to working environments, the material suggests that poor working conditions and high demands on physical and mental performance increase sick absence

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SICK ABSENCE IN DIFFERENT CATEGORIES OF EMPLOYEES

In the investigation of absence comparison between full-time and part time workers is of interest In the present material the part time salaried employees were practically speaking only females The few males working on

PART II

SOURCES OF ERROR OF SICK ABSENCE STATISTICS

CHAPTER V

THE EFFECT OF NUMBER OF WAITING DAYS ON SICK ABSENCE

It is clear from page 49 that only absence backed by a medical certificat is incl ded in the disease curves in Figs 17—18. This is a disadvantage because only about 15 % of the spells f absence are represented in this way The distribution between the different diagnostic groups would therefore probably be different if all short absences were i cluded.

Since th GSI registers contain notes of the diseases reported by its members, one might also try to distribut these among diagnostic groups It is true that it would imply an increase in the degree of uncertainty but on the other hand, most people know roughly what disease they have, .e respiratory tract disease disease of the digestive tract.

The material first tried consisted of a group of workers who were studied in Part III also for this purpose It was however found that f the 404 spells of sick absence recorded by the Company in 19"9 only "03 were registered t the CSI .e only bout 50 of the pells This was probably because it is not obligatory f r the workers to report See also page 61

It was therefore decided to tudy h w the distribution according to diagnostic groups changed f r the salaried employees who were born in 1909—1913 nd who were in the service of the Company throughout the entire year 19.9 This group consisted of ..0 men.

The result are given in Fig. "0, which in l des 3 different distributions

One gives the spells of absence of

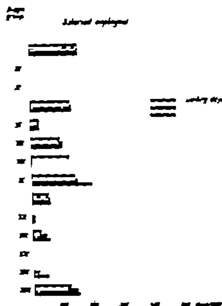


Fig 20 *Effect of number of waiting days of General Sickness Insurance upon distribution of sick absence within diagnostic groups I—XVII (See abbreviations.) Abscissa Number of days per 100 persons per year*

person who produced a medical certificate absence of more than 8 days duration. In the second all spells of more than 3 days duration and in the third all cases.

It is clear that the distributions differed particularly in group VIII (diseases of respiratory tract) A moderate increase was also found for the diseases of the digestive tract (IX) of the limbs and locomotion (XIII) and accidents (XVII) In addition the

SUMMARY

Sick absence statistics for a Company with 9 000 servants are presented and discussed

In the analysis those norms were used that are established by the International Conference on Sick Absence Statistics Special attention was given to the period prevalence rate (SAS 310) and the average duration of complete or in complete *spells observed per person* under observation (SAS 311) In addition sick absence was distributed among diagnostic groups and the importance of the different diagnostic groups in differ ent age classes was studied (See Tables 11—18 and Figs. 13—19)

The result compared favourably with data from certain other Swedish in dustries Table 20 and from literature, Tables 2 3 but there appears never theless to be room for further improv ment. Among the workers of the Company *diseases of skeleton and loco*motion of the respiratory tract, mental diseases and diseases of the digestive tract were responsible for the major part of absence Among the salaried em ployees diseases of the bones and joints were less prominent but the diseases of the respiratory tract were more im portant This was most evident on analysis of *short spells of absence*, which were due mainly to diseases of the respiratory tract.

The absence of females was twice that of males and of workers twice that of salaried employees

Sick absences (SAS 311) increases with age in males SAS 310 is influenced only slightly by age Absence of females was greatest *for those in middle age* when they presumably have most house-work.

CHAPTER VI

REPRESENTATIVENESS OF DATA FROM THE GENERAL SICKNESS INSURANCE

In th examination f effect of the number f waiting days (page 59) n the disease curves a distinct difference was found between the number of spells of absence noted in the Company books and those noted at the General Sickness Insurance This can only be explained by the a mption either that the statistics f the GSI or of the Company r both must be misleading

In an attempt to clear up this question two groups were studied.

Group 1 consisted of salaried mal employees (_50) wh had been in the Company s service t Västerås during the entire year f 19 9 nd were born in 1909–1913

Group 2 wa made up of Swedish male w rkers (*6_) who had worked in the Company in Vä terås from 19_6–19_9 and who were born in 1909–1913 These were tudied separately in Part III

What distinguished these tw groups from one an ther wa that alaried employees, but not workers, are obliged t report t the General Sickness Insurance if they re absent because of ickness. If salaried employees do not report they loss their alary from the Company during their absence For workers n wages t ll re p ea for the first 3 day of si kness unles they re ick f r 9 day more It is thus lear that w rkers ha no ad antage of reporting hort pell f absence

The following t t tical data may serve as a basis for discussion. The average duration, etc. (SAS 311) during the year 19"9 was for the salaried mployees in this age (Table 10) about 9 days and for workers 8".6 hours (See Part III Table 39) This corresponds to about 15 days f the workers.

The period prevalence rat (SAS 310) according to Table 10 is about 1.5 for employees. For the workers t was 404 cases among 26_ individuals which gives a valu f about 1.5 f them, too

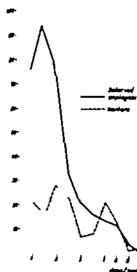


Fig. 1. Spells of sick absence of employees of same age group distributed according to duration of spells reported to General Sickness Insurance

number of uncertain diagnoses (XVI) was also increased.

DISCUSSION

It is obvious that the distribution of *absence according to diagnostic groups* with different numbers of waiting days differed in shape. This is also apparent on compilation of data from the literature (Tables 2—3).

It is evident that it was mainly diseases of the respiratory tract that were responsible for the short spells of absence of the salaried male employees. *Diseases* of the digestive tract, and of the limbs and small accidents were also responsible, though to a less extent.

It was not possible to investigate how the short spells of absence influenced the distribution for the workers since they were often not reported. But, in addition to the above-mentioned groups of diagnoses small accidents must also make themselves felt.

Neither was any analysis made of the absence of the female employees. Here one might reasonably expect an increased significance of gynaecological diseases since dysmenorrhoea usually causes short spells of absence.

Thus, profiles for diseases with a long waiting time do not give a very correct picture of the distribution of absence among diagnoses. On the other hand, it should be remembered that the longer waiting time considerably increases the reliability of a diagnosis. It is often difficult for the doctor to make a correct *diagnosis within the first 4 days of* a disease.

CONCLUSION

Although the use of distribution according to diagnostic groups with 8 days waiting time implies certain disadvantages the distributions are useful for comparisons between different groups of employees.

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In the examination of effect of the number of waiting days (page 59) on th disease curves a distinct difference was found between the number of spells of absence noted in the Company s books and those noted at the General Sickness Insurance This can only be explained by the assumption either that the tatistics of the GSI or of the Company or both must be misleading

In an attempt to clear up this question tw groups were studied.

Group 1 consisted of salaried male employees (250) who had been in the Company s service at 1 ll terás during the entire year f 1939 and were born in 1909—1913

Group 2 was made up of Swedish male w rk rs (2..) who had worked in the Company n Vll terå from 1936—19 9 and who were born in 1909—1913. These were studied parately in Part III

What d tingui hed these two groups from one another was that alaried employees but not workers are obliged t report at the General Sickness Insurance f they are absent because of ickness If salaried employees do not report, they loss thei salary from the Company d ring their absence For w kers no w ges at all re gi en for the first 3 day of sickness unless they are ick for 9 day r m re It is thus clear that workers have no advantage of reporting hort pell of absence

The following tatistical data may serve as a basis for discussion. The verage duration, etc. (SAS 311) during the year 1939 was for the salaried employees in this age (Table 10) about 9 days and for workers 87.6 hours (See Part III Table 39) This corresponds to about 15 days f r the workers

The period prevalence rate (SAS 310) according to Table 10 is about 1.5 for employees For the workers it was 401 cases among 262 individuals, which gives a value of about 1.5 for them, too.



Fig 21. Spells of sick absence of employees of same age group distributed according to duration of spells reported to General Sickness Insurance

number of uncertain diagnoses (XVI) was also increased

DISCUSSION

It is obvious that the distribution of absence according to diagnostic groups with different numbers of waiting days differed in shape. This is also apparent on compilation of data from the literature (Tables 2—3)

It is evident that it was mainly diseases of the respiratory tract that were responsible for the short spells of absence of the salaried male employees Diseases of the digestive tract and of the limbs and small accidents were also responsible, though to a less extent

It was not possible to investigate how the short spells of absence influenced the distribution for the workers since they were often not reported But in addition to the above-mentioned groups of diagnoses small accidents must also make themselves felt

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CONCLUSION

Although the use of distribution according to diagnostic groups with 8 days waiting time implies certain disadvantages the distributions are useful for comparisons between different groups of employees

CHAPTER VII

INFLUENCE ON SICK ABSENCE STATISTICS OF DURATION OF EMPLOYMENT

It is clear from the account of the sick absence (page 18) that on comparison between groups f persons who have been employed for different periods (e.g entire year compared with those who joined the Company that year) sick absence must be corrected for differences in duration of period in the Company s service Here it is discussed whether it is theoretically justified to make such a comparison, even afte correction for period of mployment

Thi chapter is concerned with the question how the period prevalence rat (SAS 310) is infl enced by the duration of employment and, secondary how the number of accidents d ring work per unit period f time is influenced by d ration of employment m the Company

INFLUENCE ON PERIOD PREVALENCE RATE

The material consisted of male em ployees in the service of the Company d ring 19 9 Of these two groups were t dird, namely those wh had w rked the entire year nd those who had joined and r left the Company that year The latter group w di ided int 6 sub-groups according t d ration f service in the Company: those who left the Company in January and February nd those who joined in Nov mber or December were pooled t form a ingle group with an a rage period of employ ment of 1 month, those who left the Company in March—April r began in September—October formed a group with duration of service of 3 months with the Company etc.

Table 21 gives the distribution per cent of those who were employed the entire year and Table 22 the distribution found f r those who were employed less than 1 year

Table 21 *Employees employed entire year distributed according to number of spells*

Number of spells	Number of employees N	%	Probability of sick absence spell of a person any month during the year
0	852	31.6	0/1
1	738	27.3	1/12
	497	18.4	/12
3	304	11.3	3/12
4	157	5.8	4/12
5	80	2.0	5/1
6	38	1 4	6 12
7	19	0.7	7/12
8	8	0.3	8/12
9	3	0.1	9 12
10	3	0.1	10/1
11			
12—	1	0.01	12/1

Table 21 includes the probability of a person being ill any month during the year It also includes the assumption that all month were equal regarding to sick absence which is erroneous but nevertheless necessary t enable later calculation (How this assumption can influence the result is discussed on page 6)

The average duration per spell (SAS 312) was thus 6 days for the employees and about 10 days for the workers Fig 21 shows that the absences of employees reported at the General Sickness Insurance and distributed according to the duration of the spells gave a curve which agreed well with the Poisson distribution, while the corresponding distribution for the workers was much more irregular On the other hand, if we consider the distribution of the absence of the workers in the statistics of the Company we see also a similar distribution curve (Fig 61)

In addition it should be mentioned that the workers wages range from 10 000 to 14 000 Swedish crowns per year so that they belong to class 11—12 of the General Sickness Insurance Judging from the analysis on page 27 it is people belonging to this class who place most claims on the GSI

Of the 404 spells in the Company's statistics only 205 were to be found in the records of the GSI

DISCUSSION

Since the distribution curve for the workers in the GSI series is irregular but that in the companies data regular it is tempting to assume that it was data on the GSI that were not complete Since the period prevalence rate in the Company's material was equally large for workers and salaried employees there is no reason to assume that the material for the workers included more cases of voluntary absenteeism

In addition, it was found that the average duration per spell was longer for workers than for employees and, judging from the experience of the Company the voluntary absenteeism is practically always very short.

It is thus probable that the workers think it hardly worthwile to report short spells of sick absence to the GSI They presumably believe that they can themselves judge how long a disease will last. That even those who are ill for a longer spell sometimes postpone reporting their illness to the GSI is not uncommon either The relatively low frequency with which workers report to the GSI is still more remarkable in the light of the fact that they belong to that group which burders the GSI most

CONCLUSION

In the investigation of sick absence for such groups in which it is not necessary for the persons to report illness to the GSI and in which such refrainment from reporting implies no economical losses it is obvious that GSI statistics reflect only a percentage of the spells of absence because of sickness This material of workers thus represents only half of the true number of sick absences In the classification of the diseases according to diagnostic group the GSI records are necessary

CHAPTER VII

INFLUENCE ON SICK ABSENCE STATISTICS OF DURATION OF EMPLOYMENT

It is clear from the account of the sick absence (page 18) that on comparison between groups of persons who have been employed for different periods (e.g. entire year compared with those who joined the Company that year) sick absence must be corrected for differences in duration of period in the Company's service. Here it is discussed whether it is theoretically justified to make such a comparison, even after correction for period of employment.

This chapter is concerned with the question how the period prevalence rate (SAS 310) is influenced by the duration of employment and, secondary, how the number of accidents during work per unit period of time is influenced by duration of employment in the Company.

INFLUENCE ON PERIOD PREVALENCE RATE

The material consisted of male employees in the service of the Company during 1959. Of these two groups were studied, namely those who had worked the entire year and those who had joined and/or left the Company that year. The latter group was divided into 6 subgroups according to duration of service in the Company: those who left the Company in January and February and those who joined in November or December were pooled to form a single group with an average period of employment of 1 month, those who left the Company in March–April or began in September–October formed a group with a duration of service of 3 months with the Company etc.

Table 21 gives the distribution per cent of those who were employed the entire year and Table 22 the distribution found for those who were employed less than 1 year.

Table 21. Employees employed entire year distributed according to number of spells

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4	157	5.8	4/12
5	80	3.0	5/12
6	38	1.4	6/12
7	20	0.7	7/12
8	8	0.3	8/12
9	3	0.1	9/12
10	2	0.1	10/12
11			
12	1	0.04	12/12

Table 21 includes the probability of a person being ill any month during the year. It also includes the assumption that all months were equal regarding to sick absence which is erroneous but nevertheless necessary to enable later calculations. (How this assumption can influence the result is discussed on page 67.)

Table 22. Employees with different duration of employment distributed according to number of spells

Average duration of employment in months	Distribution of employees according to number of spells										Total
	0	1	2	3	4	5	6	7	8	9	
11	35	11	8	10	3	1	1	1			9
9	56	28	17	3	6	5	2				11
7	99	46	16	6			2			1	172
5	75	28	14	2							119
3	168	14	5	1	1						189
1	147	5									15
											819

In an attempt to ascertain the distribution of absence of those who were employed the whole year among spells but with a study period of less than 1 year we reasoned in the following way

It is known from Table 21 that for 11.3 % of those who had been employed the whole year the probable absence for any month of the year was 3/12

If we now take any 2-month interval and wish to know how the 11.3 % were distributed among the different possibilities of absence (i.e 0 1 and 2 spells, since we only reckon with a maximum of 12 spells of absence per year) we proceed in the following way The number of possibilities of being absent 0 times in 2 months is only one i.e., he must be present the first and the second months The probability of not being absent during the two months is the probability of not being away the first month multiplied by the probability of not being away the second month i.e $9/12^2$ This means 81/144 of the 11.3 per cent of persons who were been employed the whole year had not had absence during a random 2 month period

The number of possibilities of being away once in one of the 2 months is 2, a person may be away the first and healthy the second or the other way around. The possibility of this occurring is the product of the possibility that the person will be away and the possibility of being healthy i.e (3/12 × 9/12) But this might occur in two different ways i.e the total probability will then be (2 × 3 × 9) / (12 × 12) = 54/144

The number of possibilities of being away on one occasion either month is only one and the probability of this occurring is $(3/12)^2$ = 9/144

There is also the possibility of being away once but so long as to extend into the second of the months Long absence is, however so rare that it may be ignored here

We obtained the following results

Of the 11.3 % who were away 3 times per year 81/144 were healthy 54/144 were away on one occasion, and 9/144 were away on two occasions, when we studied any two months interval

In the same way one might assess how the remaining 12 groups contribute to the variation in absence during a 2 month period If we add the probabilities for the 13 different groups for no absence for absence on one occasion etc we get a theoretical estimation of the percentage of the observed group that will fall into each category of absences

With the introduction of certain symbols we can find the formula for the calculation of the probability

a. $\binom{n}{k}$ i a symbol showing how many different ways it is possible to take out k objects from a group of n bjects. Thus, for example, $\binom{2}{1}$ = = 2, $\binom{2}{2}$ = 1 etc.

b p_i = /12 is the probability of being ill a certain month. "i" can vary from 0–1 in this case

c q_i = 1 − p_i is the probability of being healthy the month "i"

d P_i is the percentage of the total number belonging to those falling under b in categories i

e $P_{x,y}$ is the percentage f persons in a tudied interval f duration y months with x sick absences.

The f rmula for calculating the term P , would be:

$$P \quad \sum_{i \; x}^{1} \binom{y}{x} p^{x} q^{y \; x} \; P$$

the 13 different categories of frequencies re added t scertain how they together contribute to the *absence in a group tudied a certain number f months.*

With this formula we get the follow ng alues. (Since the calculation is time-consuming, especially on increase of the numbe of months, we contented our sel es with t dying the tw h rtest intervals namel 1 and 3 months.)

P 8 P 0.9
P − 1.5 P 21
$P_{2,3}$ 6.)
P − 1.3

If w con ert the percentages t whole numbers w get a direct comparison between the bsence during 1 and 3 months, respecti l f those employed the whole yea (thu the absence which hould theoretically lso hold for the hort term group) with those employed for 1 nd 3 months respecti ely

5 atrs

We get the following results:

Table 23. *Duration of employment 1 month*

Number of absences	Number of individuals	
	observed	expected
0	147	134
1	5	18

Table 24. *Duration of employment 3 months*

Number of absences	Number of individuals	
	observed	expected
0	168	134
1	11	41
2	5	12
3	2	2

W th the χ^2 test we found significant diff rences between the observed and expected values The result is given in Table 25

Table 25. *Differences between observed and expected values*

Interval	χ^2	Degrees of freedom	Significance
1 month	10.15	1	1.01
3 months	36 48	3	0.001

W thus see that those employees who were in the service f the Company for less than 1 year were away on fewer occasion than one might ha e expected a judged from those wh were in the Company for 1 year

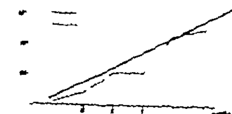


Fig 22. *Increase of period prevalence rate with observation time ( ) expected, (b) observed.*

The same thing can be seen graphically Fig 13 shows that the period prevalence rate of those employed for the whole year was independent of the age and was 1.5 spells per year For those who had been employed half a year the number of absences per individual would be 0 75 and for 1 month 0 125 Fig 22 shows how the theoretical and expected number of absences per individual increase with the observation period

INFLUENCE ON RISK OF ACCIDENTS

The number of accidents during work is widely accepted as being proportional to the period of exposure i.e the number of working hours In the study described below it was investigated whether the risk of accidents varied with the period a person had been in the service of the Company

The material consisted of one age class of male workers which included a relatively large number of persons who had been in the employ of the Company for a relatively short period, namely those who filled 20—24 years in 1959 Notes were made of the period of employment expressed in hours and the registered working hours as well as whether the workers had been absent because of accidents during the year Unfortunately it was not possible to determine the number of accidents because only some of them necessitated absence from work and secondly because only the duration of absence and not the number of spells of absence are recorded for the workers In this group that had not been employed so very long the number of individuals who had had accidents should coincide fairly well with the number of accidents responsible for absences

The results are given in Fig 23 from which it is apparent that the percentage of injured persons was greatest among those who had been employed for somewhat more than 6 months

Fig 23 also shows how many hours work had been performed per injured individual for the respective periods of employment The curve is U-shaped, which means that the risk of accidents is low during the first months it then increases continuously and is greatest after 6 months employment, after which the risk decreases Those who had been employed the entire year had undoubtedly the smallest number of accident per person

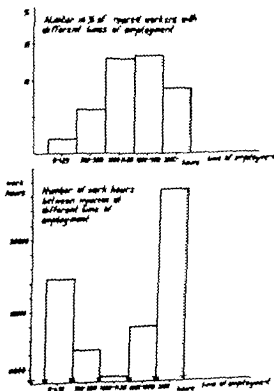


Fig 23. Relationship between duration of employment and injuries.

DISCUSSION

Both concerning the number of spells of sick absence and the number of injured individ als, distinction must be made between those who worked the entire year and those who did not

Regarding the period prevalence rate a considerabl approximation was necessary and this might have influenced the result. On p 63 it is assumed that the risk f r illness is equal all months of the year This is, however not correct According to GAFAFER (1943) and the morbid ty tatistics of the Netherland (55) absence is greatest during the first quarter of the year and then during the fourth quarter D ring the month f November—February absence is highest. This grees also with what has been found at ASFA Thi thu implies that the main part of those who had been employed f r a short time and who left the Company in the beginning of the yea or began at the end f the year were employed during those month when sickness absence mu t be expected t be highest If w mak allowance for the se sonal riation, the difference between the observed and expected absence would th be greater

W consid red only the d ration of employment and did not divide the material i t groups .e those who joined r left the Company This m ght be of some importance beca se the ss an pe f the started male employees who tarted w 59 re lower than for those wh finished According to Fig. 3 however the period prevalence rat wa tringly con t nt in the present material. According t the literature mentioned on page 0 the frequency of illness tend t decrease with age (HERMANSSON 19 4 KINLBERG 1958) while FRIBERG et al (1953) found it t incre se

The difference between those who had been employed the whole ear and those who had been employed for a shorter time could probably be explained in this way: According to the definition of the period prevalence rate (SAS 310) all absences during a certain observation period are included independently whether the spells started before or ended after the period. For those who had been em ployed the whole year all absence is thus included i.e that tarted the year before and that which was extended into the following year Those who joined the Company during the year were with but few exceptions, healthy or at least not on the sick list at the time of employ ment (All persons joining the Company are medically examined) Those who left the Company may be divided into two groups, namely those who left to tak work lsewhere and those who were pensioned off or died As to the former group it may be a umed that people do not change jobs unless they are healthy Regarding the second group data on those that died are dealt w th in a special part of this chapter (see page 68) Those who w re pensioned off because f ill health had been away from work so long that they were no longer included in the absence statistics In view of the foregoing those who joined or left th Company during the year under con ideration should have a lower frequency of spell of absence than those who had been employed the whole year

A group that has recei ed much space in the literature i that con i ting of shifters i.e indi id als who change work frequently Our finding that people who were emplo ed for only a short time were away less often from work cannot be used a n argument in the debate on hifters Those who were employed for only a short time in the present series can only to a certain extent be regarded as hifters and to compare shifters with other w rkers it is necessary either t use the point prevalence rat (SAS 110) or follow the absence of the shifters from one company to another during an

entire year and then the time lost between successive employments should also be considered.

As for accidents, investigations in Sandviken (HAGBERG 1961) revealed that the risk was *greater among beginners*, among young people and among those who had changed jobs within the Company. In the evaluation of the group of young workers allowance must also be made for the period they had been at their job. Those belonging to the short period group consisted not only of beginners but also of those who had left the Company and others who had been called up for military service that year. The U-shaped distribution (Fig 23) may possibly be explained as follows. Those who joined the Company received instruction and they must be supervised for some time and they do not feel *confident. Some months later they gain* confidence and their work need not be controlled so much, but their skill has not increased at the same rate, with the result that accidents become more common. Gradually they also became more skilled and they *realize* the importance of safety measures and instructions for the avoidance of acci*dents and the accident curve falls*. The results found in the investigation agree fairly well with the conception of the department for the protection of workers at ASEA that the risk of accidents is greatest after 3 months employment.

SUMMARY AND CONCLUSIONS

A significant *difference was found* between the period prevalence rate of male employees who had been in the service of the Company for the entire year and those who had been in the Company for less than one year. It was also found that the risk of accident *depended not only of the period of* exposure but also on the period the person had been at his job.

In the evaluation of absence a distinction should therefore be made between those who have been at their jobs for less than one year. From a practical point of view this source of error will of course depend on the *percentage of persons who have been* employed for less than one year. If the number of shifters is not too great the formula recommended by SPRATLING (see page 21) will probably be acceptable.

If different series are to be compared the observation time should be the same *for all. In some investigations* it is probably better to use the point prevalence rate (SAS 110) or the incidence rate (SAS 210) than the period prevalence rate (SAS 310). At least for ASEA SAS 310 is the simplest.

EFFECT OF MORTALITY ON *SICK ABSENCE STATISTICS*

Among people who *left the Company* during the year those who died represented *a special group*. In an attempt to ascertain how this group affects the absence statistics all those employees who died during 1959 before they reached the age of superannuation (*Table 26*) *were studied. Although* this group consisted only of 19 cases, 3 clearly different types of influence on sick absence statistics could be discerned.

a. *Sudden death without previous sick absence*. In these cases death occured before any report could be made to the GSI. *This group is thus not represented* in the absence statistics.

b. *Chronic diseases running a slow fatal course*. Most of these individual were ill already before the year studied

Table 26. Employees who died in 1959 before superannuation

Age	Disabled since	Death	Diagnoses	At work in 1959	Sick absence in 1959
Salaried employees (males)					
68	59	27.4	Haemorrhagia cerebri	117 days	0 days
43	57	10.4	Tumor cerebri	0 „	0
61	55	20.11	Infarctus cordis		0
65	58	12.5	Carcinoma bronchiale	48 „	4
46	58	12.4	Carcinoma penis	0	101
58	57	20.11	Haemorrhagia cerebri		144
58	58	9.11	Infarctus cordis	185 day 172	128
53	53	21.11	Nephrosclerosis uraemicum	762 „	62 „
35	59	7.8	Carcinoma pulmonale	19	77 „
Salaried employees (females)					
53	57	20.4	Carcinoma coli	0	0 „
Workers (males)					
63	59	15.12	Aneurysma aortae rupturans	7093 hours	0 hours
51	59	7.6	Infarctus cordis	1025	0
50	59	21.10	Aneurysma aortae rupturans	1781	0
5.	58	20.6	Neoplasma malignum renis	0	1179 „
52	58	21.	Leuchaemia	0	1221
48	5	13.8	Infarctus cordis	0	1261
50	59	28.11	Cor hypertonicum + ren cysticum bilatera-lis	2236	9
49	59	28.10	My lomatosis	1022	680
Workers (females)					
6	59	12.4	Diabetes mellitus + cystopyelonephritis	661	88

They had been pensioned ff or left the company or t n rat reached the 182nd day o the final d recommended by SAS. F r these then no w rking time si k bsence had been noted d ring the yea

These person who had had sick absence for a vary ng period before death. Therefore only group c is included in ick absence t tistics Of the 19 persons who had died, were n t awa at all because of illness were aw y for a hort period (1 and 4 days, respectively) 4 had been away the entire year until the day of death and for the remaining 6 both working time and ick absence were noted

In the curves ag is gi en a the number of f ll years before death, and onset age that age when the person wa disabled for the major part of the year by the disease (T ble 6)

CHAPTER VIII

TENDENCY OF SICK ABSENCE OF INDIVIDUAL

In the investigation of the distribution of sick absence among individuals in a given population it is not possible to dismiss the possibility of a chance distribution On comparison from one year to another of the same Company or between different Companies values found are fairly constant the time lost because of accidents does not vary much from year to year etc FORTUIN (1955) also casually mentioned that the distribution of sick absence during an observation period of sufficient duration is similar to that of a chance event (Poisson distribution) (See page 14)

Before proceeding to Part III in which sick absence is studied for any correlation with other factors it is therefore necessary to ascertain whether sick absence shows the same characteristics as chance events

The material consisted of the same persons as in Part III i.e all male workers born in Sweden in 1909—1913 and working at ASEA in Västerås and who had been employed the period 1 1.56—31 12.59 The material consisted of 262 men

The material was studied in three different ways

1 Diagrams of the distribution of sick absence spells showed only small variations from the years 1956—1959 Fig 21 shows the distribution of sick absence in 1956. Let us now check whether the distribution may be regarded a a Poisson distribution. The method used is described by CRAMER (1951)

With Cramer's symbols the Poisson distribution is

$$P(\xi = r) = \frac{\lambda}{r!} e^{-\lambda}$$

We do not know the λ for the distribution in the diagram and estimated the parameter according to

$$\lambda = \frac{1}{n} \sum_{1}^{n} x_{\mu}$$

For this distribution λ = 1 893 i.e

$$P(\xi = r) = \frac{1\ 893}{r!} e^{\ 1.893}$$

With the χ^2-distribution with r degrees of freedom we can test the significance of the differences between the observed and the estimated distribution We get $\chi^2 = 103.8$ which for 5 degrees of freedom gives $p = 0.0001$ The observed distribution can according to conventional significance level thu not be regarded as a Poisson distribution.

2 If the distribution of a certain number of spell of absence per year is x and the events are independent we can estimate the probability of the same number of spells of absence for each individual 2 years in succession with x^2 for three years in succession with x^3 etc

*On the basis of the observed prob*ability for 1956 we calculated the

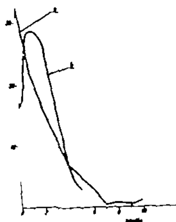


Fig. 21 Distribution of number of spells of sick absence during one year ( ), compared with the Poisson distribution (b)

$P(^{-} \quad) \quad \frac{1.893^{x}}{\quad !} \quad 1.893$

theoretical pr balal ty f r d fferent numbers f bsences during 3 and 4 consecut v years

Fig _5 gi es the observed nd theoret ical di tribution f r the category without bsence



Fig _5. Observed ( ) and expected (b) number of persons (°) without sick absence during four consecutive ears.

Fig 6 gi es the corresponding distribution for th category that were away on more than 5 occasions every year

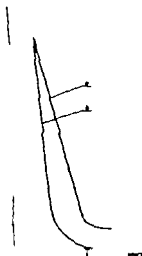


Fig 26. Observed ( ) and expected (b) number of persons (%) with five or more spells of sick absence per ear during four consecutiv years.

With the f rmula

$$t = \pm \frac{p_t - p}{\sqrt{\frac{p_t q}{n_t} + \frac{p_o q}{n_o}}}$$

where indices t and refer to th theoretical and observed distribution and where $p + q = 1$ it is possible to test the significance between correspondi g point in the two distributions

For Fig _5 w get

		point	point 3	point 4
t		3.00	4.50	4.10
sign	p	0.01	0.01	0.01

F r Fig °(we have a significant difference ($p < 0.01$) for point _ but not for point 3 and 4 which i n t surprising ince at point 3 and 4 only about 1 of the material is left

3. If the number of frequencies is due t chance there should be no correlation

CHAPTER VIII

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With the χ^2-distribution with r degrees of freedom we can test the significance of the differences between the observed and the estimated distribution. We get $\chi^2 = 103.8$ which for 5 degrees of freedom gives p 0.0001 The observed distribution can, according to conventional significance level thus not be regarded as a Poisson distribution

2 If the distribution of a certain number of spells of absence per year is x and the events are independent we can estimate the probability of the same number of spells of absence for each individual 2 years in succession with x^2 for three years in succession with x^3 etc.

On the basis of the observed probability for 1956 we calculated the

PART III

SPECIAL STUDY OF SWEDISH MALE WORKERS BORN 1909—1913

CHAPTER IX

MATERIAL

Of the Company s servants only those satisfying the following criteria were accepted for thi part of the investiga tion

1 Sex: males
2 Age born 1909—1913
3 Nationality and environment during hildhood. nly Swedes born in Sweden
4 Working place in ASEA work hops at Vä terås.
5 Occupation. w ker
6 Period of employment: uninterrupted employment a w rker from 1 1.56 31 1 .59 at ASEA.

The series con i ted of *6 men

The crit ria gi en abov were selected for the following reason

SEX It is known that ick bsence differs with sex (see P rt I) therefore in the search for an answer t the question posed, the nvestigation hould, at least in the beginn ng be lim ted t one sex. Males were hosen f the present invest gation because the number of female work rs in ASEA w not large enough to allow of tisfactory tatistical treatment

AGE It is well known that sick absence aries with ge In order t eliminat this source f error without undue reduction of the si of the material, ge cla ses were selected a h is These age classes were chosen for the following reason

Diseases ascribable t environmental factors, such as noise injuries, pneumoconioses alcoholism, had required many years to develop ften more than *0 years. (Thi of course does not apply to acute industrial injuries.) Manifest diseases due t environmental conditions can therefore hardly be expected until after the age of 40—45 years

On the ther hand, one hould not wait until such diseases have become so serious that a worker ha left the Company because of ill health.

The selection of such age-classes enables an after-examination of the same persons 5 10 and 15 years before they are entitled t an ld-age pension.

NATIONALITY AND ENVIROMENTS DURING CHILDHOLD— In rder to avoid misunderstandings wing t difficulties w th the Swedish language and to facilitate description of environment during child hood, only Swedes wh had grown up in Sweden were accepted

OCCUPATION — Workers were chosen because it is easier to judge their perf rmanc and the demand placed upon them by thei work. In addition it provided the pos ibility of using the record kept by the Company f their general behaviour and performance

WORKING PLACE — Since it wa necessary also to study the working places only workers employed in the work hops or f ctories in Västerå were included. Field assemblers belonging to the Vä terås factories were thu not taken int account

between the number of spells of absences for the individuals in different years

The product moment correlations between the 4 years studied are given in the following list (conditions for product moment correlations are not entirely satisfied but precision is not necessary for interpretation of the results)

	1957	1958	1959
1956	589	498	494
1957		.553	457
1958			.523

It is thus obvious that during the 4 year period studied those who were frequently absent one year tended to be so also in the other years

All 3 analyses set forth above showed the same thing namely that the distributions of the sick absences found were not due to chance

CHAPTER X

METHODS

As mentioned in the introduction, the investigation was based as far as possible on data available in different registers.

The probands included in the series were approached in the following way

The various workshops were visited by the author together with a time-motion engineer (Eng. K. Hernod) and the working environment were studied and data on the workshop collected. On that occasion the workers were invited to take part in the investigation. The workers were informed that the purpose of the investigation was to study the general state of health and that all information obtained would be treated as confidential and that it would be codified by the author himself. If a worker hesitated to participate, he was informed that participation was voluntary but that the investigation would be of value not only to the author and the Company but also to himself as well as to the community. Apart from this no attempt were made to persuade the workers to cooperate. It might be mentioned that persuasion was not necessary because all except 4 who refused to cooperate were only too willing to join the investigation.

The probands were then called to ASEA health centre where they were interviewed and examined clinically by the author. In order not to bias the author's examination of the probands, the data obtained from the local temperance committee for example were not noted for each proband until after the interview. After the interview the proband's absence card was studied for supplementary data of historical interest. The probands cooperated so well that it was seldom necessary to correct their reports by data obtained from other sources. The interview was kept in as free a form as possible though more or less in accordance with a questionnaire that was completed in the course of the interview. The interview and clinical examination took about one hour per individual. On a later occasion the proband was again called to the health centre for anthropological examination and assessment of his physical performance. This examination required about hours.

At the clinical examination the proband was given a form to fill in concerning consumption of alcohol, etc., during a working week. When he was given the form, he was informed that he need not fill it in if he did not wish to. The workers received such a form on one occasion only.

STATISTICAL METHODS

Owing to the size of the material (*6.. probands and 190 variables) the analysis of the data would not have been possible without an electronic data machine (Facit EDB). The treatment of the data was briefly as follows.

The material was built up according to Matrix I

PERIOD OF EMPLOYMENT

In the choice of period of employment in the Company it was decided to have as long an observation time as possible without unduly encumbering the statistical treatment of the data. By following the absence for a 4-year period, it was possible to compare the sick absence from one year to the other and to obtain more detailed information on the diseases responsible for absence than would have been possible for a shorter period.

As mentioned, 262 workers satisfied the above mentioned criteria Of these, 4 refused to cooperate and 6 others could not take part in the investigation Complete data were not available for all the variables of all individuals The number of individuals, for whom data were available on each variable will therefore always be given.

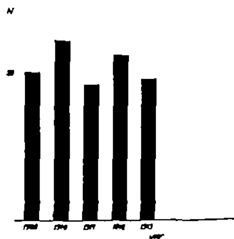


Fig 27 *Distribution of probands according to year of birth*

CHAPTER XI

SOCIO MEDICAL VARIABLES

This hapt r is concerned w th social and medical ariables that can probably be correlated with k absence

The environments of industrial w rk ers hav been tudied fairly thoroughly in some ind trial districts in Sweden (SEGERSTEDT & LUNDQUIST 193.., 1955) Large numbers f people ha been int rviewed. Attention ha been given t the actual cond tion in the w rkshops t nd during non-working hours as w ll a t the social att tudes It would hav been v la ble if this n t m could ha been coped n the present in estigation. e the ame riables but thi would b been less purposeful Therefore in those respect in which data could not be btained direct from the regi ters riables suited t the present n est g tion were con tructed.

CHILDHOOD ENVIRONMENTS

This section deal w th some ariables illu trating env ment l conditions be tween the ges of 0 15 y rs The dat w re bt ined from interview with the p band Th re w no pos ibility of becking the relial lit f the data obtained There f course the ri k that the mem ry the proband had of thei hildhood h d been coloured b impression l t n lif In addition, some of the riables were difficult t judge F alu tion wa based on a d scu ion bet een the uthor and the proband If n ubstantial changes had occurred concerning any ariable during the period 0 15 ars f age the variable studied was given the grade that had the longest duration.

Mean values of the different variables are given in Tables 26, ..

FAMILY CONDITIONS — The material w di ided into 6 classes (Fig 28)

a) Grown up in parents home Both parents li ing and dwelling together

b) Grown up in good foste home

) Crown up in parents hom but father or mother dead before proband wa years of age

d) Brought up by only on of the parents because of di orce separa tion

) Brought up by unmarried mother

f) Brought up in a hildren s hom

SOCIO-ECONOMIC STATUS (Fig 28)

The material wa divided into 3 grades A certain amount of overlapping w unavoidable The material wa divided in the following way Those brought up in 1910–192. in a n rmal Swedi h w rker family where the father wa li ing and u ually had work, were gned to Grad Those with a lower standard, g the mother alon the father often unemployed or large number of ilo (m re than 10) chronic disease f ignificance in the family etc., were a gned to Grade 1 Those with better standard, i.e., father a white-collar w rker r farm owner or bu ines wner were a signed t Grade 3

Matrix I	Proband No.	Variable 1		3	4	5	6	N
	1	x_{11}	x_{12}	x_{13}	x_{14}			x_{1N}
	2	x_{21}	x_{22}	x_{23}				x_{2N}
	3	x_{31}						
	4							
	5							
	n	x_{n1}	x_{n2}					x_{nN}

The *strips for Facit EDB* were stamped in row vectors i.e the variables *for each proband* were stamped in a consecutive series

For each column vector i.e each variable, the arithmetic mean and the standard deviation were calculated in the conventional way

In the calculation of the correlation between different variables, the column vectors were first normalized so that the median md_j was determined for each column vector j and all $x_{ij} \leq md_j$ gave the value 1 and all $x_{ij} > md_j$ gave the value 2

All the *observations for pairs of* column vectors were stored, e g j and k in a four-squared field according to matrix II pairs of observations with a value of 2 for j and 1 for k being stored in square a etc Chi square was then calculated according to the following formula

$$\chi^2 = \frac{n\,(bc - ad)^2}{(a+b)\,(c+d)\,(a+c)\,(b+d)}$$

Since the difference between the diagonal products in matrix II was squared, it was not known whether ad or bc was greater which was of interest for interpretation of the result since the correlation was positive if bc was greater than ad and vice versa The machine was therefore set so that it *plus if bc was* greater than ad and minus if bc was less than ad In addition, when writing out the results only those correlations were included for which the Chi square test gave 3.84 or more which with one degree of freedom gives $p < 0.05$

Matrix II		k: 1	
j		*a*	*b*
	1	*c*	*d*

The χ^2 method cannot always be used This applies to the first of the variables where the numerical values give no information Secondly χ^2 gives information only if the relationship in a given case is linear If the distribution is instead U-shaped, which in this case would mean that the absence is lowest somewhere near the mean of the variable the χ^2 test is of no value Thirdly χ^2 *requires* that the distribution of the material in which the variables are *studied must be normal.*

χ^2 analysis was therefore not enough it was necessary *to distribute the* material among the different degrees of the variables and calculate *the mean for each degree of variable* The significance of the differences found between absence for the different degrees of variables were *then tested by the t test*

This procedure also gave the distribution *of each variable* which proved necessary complement to the mean and the dispersions

CHAPTER XI

SOCIO MEDICAL VARIABLES

Thi ch pter i concerned with social and medical variables that can probably be correlated with sick absence

The environment of indu trial w rk r has been tudied fairly thoroughly in some indu trial district in Sweden (SEGERSTEDT & LUNDQUIST 195_ 1955) La ge numbers f people hav been interviewed Attention ha been gi en t the actual conditions in th w rkshop etc and during n n working hours a w ll t the social att tudes It would ha been lnable if this s tem could hav been copied n the present in- est gation e the same ariables, but thi would ha been les purposeful. Therefore n those respect n which data could not be bt ned d rect from the regi ters ariables omitted t the present in est gation w re onstructed

CHILDHOOD ENVIRONMENTS

This section de l w th some ariables illu tr ting en ronment l cond tions be tween the ges f 0 l rs The d ta were btained f om int rview w th the proband There w no pos ibility f hecking the reli b lit f the d t obt ined There f ourse the ri k th t the memory the p band had f thei hildhood had been coloured by impression lat n lif In ddition som f the riables were difficult t judge F lu t n w based on discussion bet een th uthor and the probands If hei ntial hanges had occurred oncerning n ariable during the period 0 l ears of age the ariable tudied was given the grade that had the longest duration

Mean values of the different variables are gi en in Tables 6, 27

FAMILY CONDITIONS — The material w divided into 6 cla ses (Fig. 28)

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) Brought up by unmarried m ther
f) Brought up in a children s home

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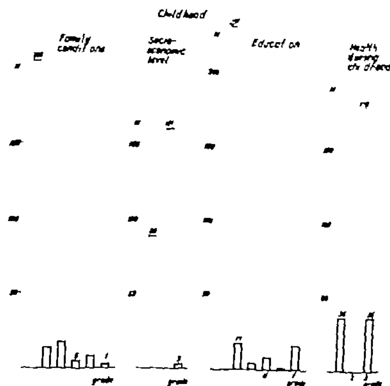


Fig 28. *Distribution of probands according to variables during childhood.*

Table 26. *Childhood environments (Means and standard deviations)*

	N	M	S
Family conditions	5	See Fig. 28	
Socio-economic level	45	1.7	0.5
School education	451	See Fig. 28	
Health during childhood	251	See Fig 28	
Size of town or village during childhood	452	12160	337 8

SCHOOL EDUCATION (Fig 28)

Most of the men left school before they were 15 years of age Some of them, however took evening classes later It was not considered necessary to treat this variable separately

a) Six years ordinary elementary school
b) Elementary school plus 1—2 years in continuation schools
c) *Peoples high school* One or 2 years course
d) Some occupational school *e.g.*, work shop *school*
e) Taken the general school examination.
f) Taken evening courses or correspondence courses in the evenings after they had finished their work.

HEALTH DURING CHILDHOOD (Fig 28)

Three-grade scale

1 Those who were seldom or never ill.
2. Largely healthy except for infectious diseases men les scarlet fever chicken pox, etc
3 Delicate children who often had infection or colds many diseases of childhood or complication those who had rachitis or poliomyelitis (many diseases of childhood or subsequent complication such a rachiti or poliomyelitis)

The last two variables may as mentioned, be influenced by variou irrelevant factors during childhood or later *But the variables do at any rate show* whether the proband remember their childhood as a fairly bright or fairly *gloomy period*

Fig. 29 Geographical distribution of probands during childhood.

SIZE OF HOME TOWN OR VILLAGE

The ize of town re gi en according t thei population in about 19?0 For those wh grew p in the country the ize f the llage f rm gi en the numbe of nhab t nts The number thu aried between 10 nd 3 000 (Stockholm) The alues were fed int F rit FDB nd treated in log rithmic scale (F g 29)

SITE O PLACE WHERE PROBANDS WERE BROUGHT UP

The material w divided int districts Vä terå however recorded sep rat ly (F g. 29)

PARENTS AND SIBS

It w con idered des ble to btain information also on the parent nd sibs of the proband It wa originally intended t analyse the health of the relat es and auses of death. The inf rmation gi en on the health of the parents and ibs however wa very vague It was therefore decided to limit the investigation to the variables given below It was also decided to abandon the idea of determining the causes of death in the parish registers because among other things it would have been so very time-consuming, and it was nly in recent years that it has been necessary f r doctors in the country t issue a death certificate

If father s still l ving present age on 31 1?.59 (Fig. 30)

If father i dead age at time of death (Fig 30)

If mother i l ving age on 31 12.59 (Fig 30)

If mother i dead age at tim of death (Fig 30)

Fig 30 Distribution of probands according to parents.

Number f sibs The pr band is in luded so that the smallest numbe f sibs wa one (Fig 31)

Number f sibs who had died This variable depend on the numbe of ibs the proband ha or had had. It wa however con idered sufficient to n te the number f death These data are founded entirely on information given by the proband There i hardly any

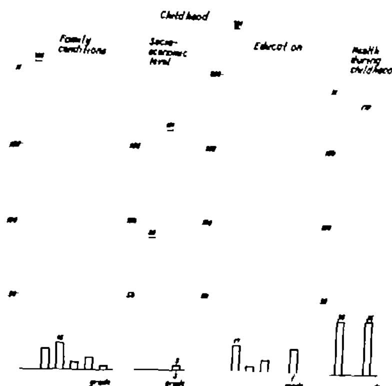


Fig 28. *Distribution of probands according to variables during childhood*

Table 26. *Childhood environments (Means and standard deviations)*

	N	M	S
Family conditions	252	See Fig 28	
Socio-economic level	252	1 7	0.5
School education	51	See Fig. 28	
Health during childhood	251	See Fig 28	
Size of town or village during childhood	25	12160	33778

SCHOOL EDUCATION (Fig 28)

Most of the men left school before *they were 15 years of age Some of them* however took evening classes later It was not considered necessary to treat this variable separately

a) Six years ordinary elementary school
b) Elementary school plus 1—2 years in continuation schools
c) Peoples high school One or 2 years course
d) Some occupational school, *e g.*, work shop school
e) Taken the general school examination
f) Taken evening courses or correspondence courses in the evenings after they had finished their work.

HEALTH DURING CHILDHOOD (Fig 28)

Three-grade scale

1 Those who were seldom or never ill.
2 Largely healthy except for infection diseases measles scarlet fever chicken pox, etc
3 *Delicate children who often had* infection or colds, many diseases of childhood or complication those who had rachitis or poliomyelitis (many diseases of childhood or subsequent complications such as rachitis or poliomyelitis)

The last two variables may a mentioned be influenced by various irrelevant *factors during childhood or later* But the variables do at any rate show whether the proband remember their childhood as a fairly bright or fairly gloomy period

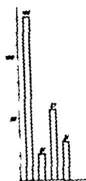


Fig 33. Distribution of probands according to National service

b) less good state of health (National service C. 3)

c) exempted from national service on medical ground

d) exempted, on other grounds from national service during peace time

Workers in the present material did their national service between the years of 19?9—1933 The military training centres at that time were fairly limited in Sweden and could not accept all of the young men who were liable to military service Therefore some were exempted even though their health was good. During W rld War II many of these men were however called up for national service On the other hand, the health f those who had been given a clean bill of health might ha e hanged in the course f years. These *changes were disregarded so that only* the tat of health at the time of enrolment f national service was considered

PRESENT ENVIRONMENTAL VARIABLES

FAMILY CONDITIONS

CIVIL STATUS (F g 34) Ci il tatu on 31 1.-59 wa n ted a follow

) married d) w dow

b) rem rried) unmarried

) d orced

It hould be bserved that the official tatu does n t al y correspond t the true civil t tu f man f the unmarried men had been living t gethe f r several rs w th their fiancée housekeeper II wev only the off cial tatu is on d red

HEALTH OF WIFE (Fig 34) Inf rmat n w requested f ll of those who were ffici lly m rried It w possible t btain an bject measure f the tat f health of the w es by tudying the registers of the (.S.I nd then add ng the number of sick d d ring the 195 19 9 It w found that f the 01 w es many 1.3 (61) had not been on the sick li t f the (.S.I t all during the I y a It ob ous that this does not correctl reflect the t t f the health, and it upports the opinion given in P rt I on p 62 It is however probble that those women who had been *ill* for longer period had reported to the G.S.I If the health f the wif has any influence on the tate of health of the hu band, it hould be reflected by those who had been ill for a long time

NUMBER OF CHILDREN (Fig 34) The In f rmation on this point was requested whether the men were married or unmarried

NUMBER OF CHILDREN WHO HAD DIED (F g 34)

LIVING ACCOMMODATION

The izes f the flat or houses are given per number f room in Fig 35

FLOOR SPACE (Fig 35) Many f the men did not know the floo space of their homes with certainty but since most of them li in tandard types f flat it was a to btain the information. For those hiring a furnished room, the ize of the room wa noted. The ize w registered in m²

6 ar ?

reason to doubt the correctness of the data (Fig 31)

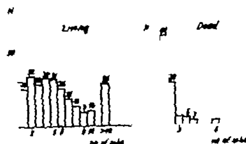


Fig 31 *Distribution of probands according to number of sibs.*

Table 27 *Means and standard deviations of parents home state of health until 1956 and National service*

	N	M	S
If father living present age	68	69 years	5,1
If father dead, age at death	170	64 7 „	15 1
If mother living, present age	105	74 8 „	5 7
If mother dead age at death	144	64.5 „	14.9
Size of sibship	252	5.3	3.4
Number of sibs who have died	252	0 7	1 3
State of health from 15 years of age until 1956	252	See Fig 32	
National service	252	See Fig 33	

STATE OF HEALTH FROM 15 YEARS OF AGE UNTIL 1956

It is difficult to obtain an objective measure of the state of health from adolescence until the year 1956 In this respect, the investigation was based only on the information given by the proband at the interview The proband were however questioned not only regarding their state of health in general but also whether and how often they had consulted the family doctor and whether they had ever been admitted to a hospital It is probable that some diseases had been forgotten or by the probands believed to be of so small interest that they were not mentioned

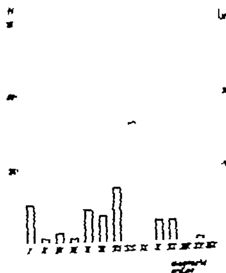


Fig 32 *Number of probands with disease any time from 15 years of age until 1956 distributed according to diagnostic groups I – XVII in real numbers and per cent.*

This error holds e.g., for diseases in upper respiratory tract The diseases reported were distributed among diagnostic groups (WHO s classification)

Fig. 32 gives the number of proband in per cent that had had some disease in the respective diagnostic groups But no information is given on the number of times the patients had had the disease or on the duration of sick absence In the treatment of the data in the Facit EDB machine an attempt was made to assess this quantitatively by noting how often the disease had occurred The duration of the disease was not taken into account unless it had lasted several years and then it was noted as the number of years the proband's disease had reduced the proband's working capacity by at least 50

NATIONAL SERVICE

The national service of recruits affords a good picture of their state of health at 20 years of age The following grading was used (Fig 33)

a) fit for national service (A 1)

FLOOR SPACE PER INDIVIDUAL (Fig. 33)

This could be readily calculated from inf rmation on the size of the flat and the number of people li ing in it.

The mean values of th variables are given in Table 28

Table 28. *Family Conditions (Means and standard deviations).*

	N	M	S
Ci il status	25	See Fig. 31	
Health of if	701	19.1 days	92.1
No. of children	23	1.3	1.6
No. of children ho hav died	182	0.8	0.1
Size f flat in rooms incl kitchen	218	2.9	0.9
Floor space of flat	48	51.0m²	18.2
Floor space per person	18	70.6m²	8.0

LEISURE

ATHLETICS In rder t f rm an opinion of the phy ical exercise of the group examined, the foll wing variables were chosen Sport (F g 36)

A three grade sc le was con tructed.

1 N phy ical exercise in the f rm of any port at all.

Formerly tiv port man but not during la t 4 ears

3 A t port man also during la t 5 ears

OPEN AIR PASTIMES (Fig. 36) Here too, a three grade scale was constructed. Activity in the open air during working hours or on the way to and from work was not included.

1 Seldom outdoors during free time.

2 Takes an evening walk or a Sunday walk fairly regularly to get some fresh air

3 Often out in the woods some doing gardening etc.

INDOOR PASTIMES (Fig 36) This variable covers activity mainly indoors. The term hobby does not cover all that is to be understood as activity indoors because active w rk in club lif f r example is valued high. The grading will be clear from the following examples

1 Those who take hardly any initiatives: *Listen to radio or look at TV* go to the cinema n w and then and read the newspaper

2 In addition to the entertainment under 1 they read book look after thei auto-cycle motor cycle car and d imple w rk at home etc.

3 Those who hav some hobby e.g., of artistic nature e.g., painting, sculpturing, membership of choir or singing lub collecting stamps antiqui-

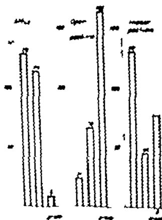


Fig 36 *Distribution of probands according to activities during free time*

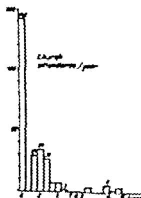


Fig 3 *Distribution of probands according to church attendance* (See page 81.)

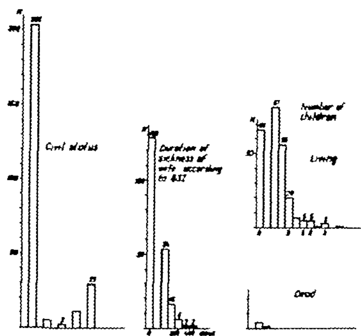


Fig 34 Distribution of probands ccord ng to f mily ond tions.

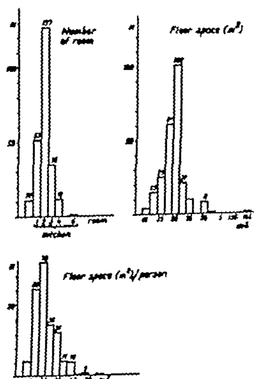


Fig 35 Distribution of proband according to l ving accomodation

FLOOR SPACE PER INDIVIDUAL (Fig 35)
This could be readily calculated from information on the size of the flat and the number of people living in it.

The mean values of the variables are given in Table 28

Table 28. *Family Conditions (Means and standard deviations).*

	N	M	S
Civil status	252	See Fig. 34	
Health of wife	201	39.3 days	92.1
No. of children	252	1.5	1.6
No. of children who have died	182	0.0	0.1
Size of flat in rooms incl. kitchen	248	2.9	0.9
Floor space of flat	248	54.0m²	18.2
Floor space per person	248	20.6m²	8.0

LEISURE

ATHLETICS In order to form an opinion f the physical exercise of the group examined, the following variables were chosen. Sport (Fig. 36)

A three grade scale was constructed.

1 N physical exercise in the form of any sport t all.
2 F rmerly active sportsman but not during last 4 years.
3 Active sport man also during last 4 years

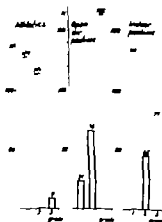


Fig. 36. *Distribution of probands according to activity during free time*

OPEN AIR PASTIMES (Fig 36) Here, too, a three grade scale was constructed. Activity in th open air during working hours or on the way to and from work was not included.

1 Seldom outdoors during free time
2. Takes an evening walk or a Sunday walk fairly regularly to get some fresh air
3 Often out in the woods, some doing gardening etc.

INDOOR PASTIMES (Fig. 36) This variable covers activity mainly indoors The term hobby does not cover all that is to be understood as activity indoors because active work in club life, f r example, is valued high. The grading will be clear from the following examples.

1. Those who take hardly any initia tives: Listen to radio or look t TV go to the cinema n w and then and read the newspaper

In ddition to the entertainment under 1 they read books, look after their auto-cycle, m tor cycle o car and do simple work at home, etc.

3 Those wh have some hobby *e.g.*, of artistic nature g., painting sculpturing, membership of choir or inging club, collecting stamps, antiqui-

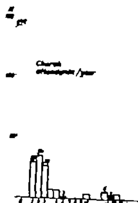


Fig. 37 *Distribution of probands according to church attendance. (See page 84.)*

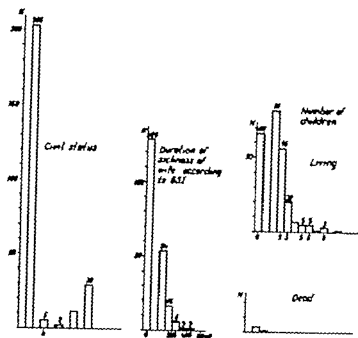


Fig 34 Distribution of probands according to family conditions.

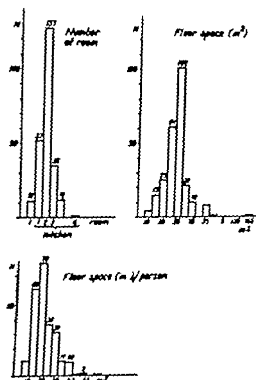


Fig. 35. Distribution of probands according to living accomodation.

FLOOR SPACE PER INDIVIDUAL (Fig 35)

This could be readily calculated from information on the size of the flat and the number of people living in it.

The mean values of the variables are given in Table 28

Table 28. *Family Conditions (Means and standard deviations).*

	N	M	S
Civil status	252	See Fig. 34	
Health of wife	201	19.3 days	72.1
No. of children	252	1.3	1.6
No. of children who have died	182	0.0	0.1
Size of flat in rooms incl. kitchen	248	2.9	0.9
Floor space of flat	248	54.9m	18.2
Floor space per person	248	20.6m²	8.0

LEISURE

ATHLETICS In order to form an opinion of the physical exercise of the group examined, the following variables were chosen. Sport (Fig 36)

A three grade scale was constructed.

1 No physical exercise in the form of any sport at all.

Formerly active sportsman but not during last 4 years.

3 Active sportsman also during last 4 years.



Fig. 36. *Distribution of probands according to activity during free time.*

OPEN AIR PASTIMES (Fig. 36) Here, too, a three grade scale was constructed Activity in the open air during working hours or on the way to and from work was not included

1 Seldom outdoors during free time.

Takes an evening walk or a Sunday walk fairly regularly to get some fresh air

3 Often out in the woods, some doing gardening, etc.

INDOOR PASTIMES (Fig 36) This variable covers activity mainly indoors. The term hobby does not cover all that is to be understood as activity indoors because active work in club life, for example, is valued high. The grading will be clear from the following examples

1 Those who take hardly any initiatives. Listen to radio or look at TV go to the cinema now and then and read the newspaper

2 In addition to the entertainment under 1 they read books, look after their auto-cycle, motor cycle or car and do simple work at home, etc.

3 Those who have some hobby e.g., of artistic nature, e.g., painting sculpturing membership of choir or singing club, collecting stamps, antiqui-

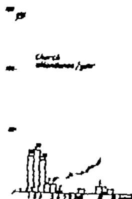


Fig. 37 *Distribution of probands according to church attendance.* (See page 81.)

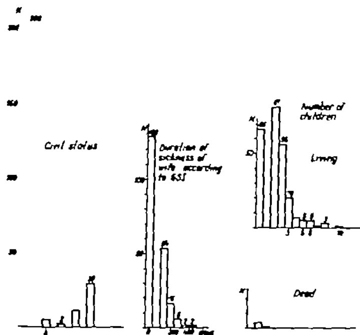


Fig. 34 Distribution of probands ccording to family conditions.

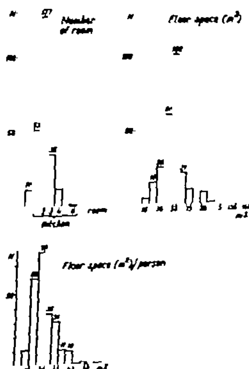


Fig 35. Distribution of probands accordi g to living accomodation.

the United States (FAO), for example has given calorie requirements for a reference man and a reference women in temperate clim te An age is 25 years and a weight of 65 and 55 kg., respectively and moderate physic activity are supposed. The calorie requirements are calculated at 3,200 for males and 2,300 for females. These figures decrease by about 3 % pe decad between 30—50 years. FAO have also worked out a correction formula f age and body weight (ABRAMSSON 1959)

General instructions fo the increased calorie requirements of people doing physical work can hardly be gi en (ABRAMSSON) Various diff rent occupa tions have been studied. ZOTTERMAN & LUNDGREN 1946, f example, calculated the requirements f f rest workers in Sweden as about 5,500—6,600 kcal/day

The ptimal composition of the diet has also been the subject of intense tudy In Sweden attempts have been mad t increase the consumption of fruit and vegetables in the diet and to decrease the amount of animal f t. For the *present investigation, however* it was sufficient t form a general opinion of the dietary habits of the probands It was n t always possible to record the con sumption in detail, mainly because f la k of time Neither was it consid red necessary t measure the calorie intake because body fat was measured. According t BROZEK (1956) the amount of body f t is a good measure of cal ric intake during long period.

Data on dietary habits collected partly from the oral interview partly from questionnaires covering the con sumption of week (see p) Information w requested on the con umption f drinks, which was easy t not and the number f times per day the proband eat meat, pork, fish, egetables fruit, etc. Only those who filled in this questionnaire form the basis of the variables

Variables

General evaluation of dietary habits (Fig 40)

a) poorer family food than usual.
b) ordinary family food.
) better than ordinary family food
d) vegetarians.

Those who had not regular meals and did not eat a warm dish with meat r fish at least 5 times a week, were placed under subheading a) Those who had a warm meal at least 5 times a week with meat or fish, were assigned to group b) Vegetables or fresh fruit in ddition to potatoes were not consumed daily Those who had ordinary family food plus fresh fruit o greens (conserved r fresh) were assigned to group c) Group d) consisted of vegetarians.

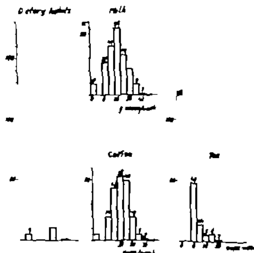


Fig. 40. *Distribution of probands according to dietary habits and consumption of beverages.*

The consumption may be influenced by the season and hereby introd ce a source of error because the investigation co ered half a year Since the interviews were carried out from December to May only conserves or imported good were av ilable N waday the prices of goods

ties, etc Those with some office in a club are also assigned to this group

RELIGION (Fig 37) No attempt was made to get deeper insight into the attitude of the group towards religion It was only desired to get a simple measure of whether they were regular church goers or not during 1959 Listening to sermons on the wireless or TV was not included On the other hand, church going was included if they reported I generally accompany my wife or if they pointed out that they went to church mainly because they were members of the choir The number of times they attended divine service was thus not a true measure of religious feeling but rather of their religious habits Of those who reported that they went to church regularly one was a Roman catholic (accompanied his wife) the others were protestants and either went to Swedish church or some free church

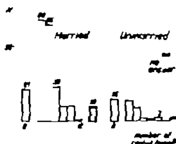


Fig 38. *Distribution of probands ccord ng to sexual life*

SEXUAL LIFE (Fig 38) Sexual life was measured by frequency of coitus per month This question was only included with hesitation because it was not known how the probands would react Most of them, however considered the question justified and gave prompt replies Those, who hesitated, however were informed that they were not forced to answer Only 11 refused. Some reported that the frequency was not due to themselves but often to the health and attitude of the wife The series was divided into married and unmarried

SLEEP REQUIREMENTS (Fig 39) Sleep requirements also reflect the way in which a person spends his leisure time The sleep requirements are given in hours per day



Fig 39 *Distribution of probands accord ng to sleep requ rements.*

T ble 49 *Indoor and outdoor activity during free time*

	N	M	S
Sport	5	See Fig	36
Open ir pastime	–5		36
Indoo pastime	–5		36
Religion	15	6	8.0
Sexual lif	41	3.1	–6
Sleep requireme t	18	1	1.6

CONSUMPTION

DIETARY HABITS

It is well known that the amount and composition of food is essential to health Attempts were made to calculate the necessary numbers of calories The *Food and Agricultural Organization of*

ment (N tional Social Welfare Board of Sweden)

Sale of all alcohol is also in the hands of State Company (*Nya System AB*) This company sells all drinks stronger than bee class II The sale is controlled by th Control Board (*Kontrollstyrelsen*) and is responsible to th Department of Finance.

DEFINITIONS OF ABUSE OF ALCOHOL AND ALCOHOLISM

WHO suggests the following definitions to define the terms "excessive drinking" and " lcoholism"

Excessive drinking is any form of drinking which in its extent goes beyond the traditional and customary "dietary use, or the ordinary compliance with the social drinking customs of the whole community concerned, irrespective of the etiological factors leading to such behaviour and irrespective also of the extent to which such etiological factors are dependent upon heredity constitution, or acquired physiopathological and metabolic influences.

Alcoholics are those excessive drinkers whose dependence upon alcohol has attained such degree that it shows noticeable mental disturbance or an interference with their bodily or mental health, their inter-personal relations, and their smooth social and economic functioning; or who show the prodromal signs of such developments. They therefore require treatment.

In his monograph "The Disease Concept of Alcoholism" JELLINEK 1960 showed that the term alcoholism is defined in different ways and that the concept of the term varies widely H found this t be natural since the habits in different countries differ so widely and he suggested the use f the following definition. Alcoholism is any use of alcoholic beverages that causes any damage to the individual or society r both.

This definition is rather vague and JELLINEK theref re gives definitions of five species f alcoholism

In Sweden the term abuse of alcohol and alcoholism are common.

In the investigation f *Nykterhets kommittén* the t rm abuse of alcohol is defined according to the data available at the authorities watching over the use and abuse of alcohol and is divided into 3 groups. Group 1 includes those who have been taken care of by the community according to § 1 of the law of abuse of alcohol during the years 1943—1945 To Group 2 are assigned those who have had at least 3 convictions for drunkenness or for other misbehaviour under the influence of alcohol during the years 1936—1945 and Group 3 includes persons who, on some occasion or another were reprimanded by the temperance board in 1936—1945

ABUSE OF ALCOHOL IN SWEDEN

Of the men above 15 years, Group 1 consisted of 0.57 %, Group 2 of 1.32 % and Group 3 of 5.82 %, i.e., a total of 7 7 %. In the t wns the total of the 3 groups was 10.6 % For the women in the whole of Sweden, it was 0 17 % and in the towns 0.32 %

ESSEN MÖLLER et al. (1956), who studied a provincial community found that 13.5 % of the adult population abused alcohol or were still abusing it. According to ESSEN MÖLLER (personal communication) the same definition was used as KALI (1960) in his account, i.e., abusers are heavy consumers and chronic alcoholism is characterized by 1) a pathological desire for alcohol after ingestion of small quantities, 2) regular black-outs during intoxication with alcohol, and 3) a physical dependence on alcohol. KALI stressed the difficulty in distinguishing alcohol abuse from normal drinking and therefore divided his material into 5 groups according to drinking habits, as noted in the registers of the temperance board.

The number of persons, who had been convicted for drunkenness in 1954 because of drunkenness in Sweden, and which is usually taken as a measure of the abuse of alcohol was 7.8 per thousand

are fairly steady and the supply is fairly constant all the year round As mentioned, only weeks without general holidays were considered so that the values noted were not influenced by holidays, such as Christmas or Easter

Notes were made of the following beverages (concerning alcoholic drinks, see below) milk, coffee and tea

The dietary and the drinking habits (excluding alcohol) are apparent from Fig 40 and Table 30

Table 30 *Dietary and drinking habits (Means and standard deviations)*

	N	M	S
Evaluation of dietary habits	195	See Fig. 40	
Cups of coffee/week	195	26.5	13.7
Glasses of milk/week	195	15.9	9.6
Cups of tea/week	195	2.7	

ALCOHOL

It is well known that abuse of alcohol can impair health The consumption of alcohol was therefore studied for any effect it might have on sick absence

HISTORICAL

Drinking habits vary widely from country to country (WHO 1951) Comparisons of the consumption of alcohol in different countries is therefore difficult. As to Sweden, data are available from statistical studies performed in 1944 by *Nykterhetskommittén*, Study in Alcoholism by ÅMARK (1951) The alcohol Question in Sweden (1960) *Blå boken* in 1951 and The White Book on the question of alcohol in Västerås (*Vitbok i alkoholfrågan i Västerås*) (1961)

In the beginning of the 19th century the consumption of alcohol in Sweden was very high It was assessed about 1830 when the temperance movement developed in this country to have been about 46 liters per year per capita or about 1/3 of a liter of spirits per adult per day In 1917 the purchase of spirits by individuals was rationed by means of a ration book (*Bratt systemet*) This book entitled the owner to purchase 1—4 liters per month Only persons over 21 were granted a ration book, which was withdrawn in the event of abuse of alcohol. Nevertheless (or thanks to this system), the consumption increased continuously and did not prevent alcoholism among youths In 1955 the ration book system was abandoned In order to prevent individual profit by the sale of alcohol or spirits, the sale was monopolized by a State Company and attempts are being made to control the consumption of alcohol by taxation. The tax on spirits today is about 85 % and on beer class II, 35 %

The consumption of wine and beer in Sweden has always been relatively small compared with that of spirits Consumption of spirits is high and since the abandonment of the ration book, it is probably the highest in the world In recent years, however the consumption of wine has increased at the expense of spirits. The consumption per person in 1959 is given in Table 31

Table 31 *Consumption of alcohol per inhabitant in 1959 in Sweden*

	Amount (Liters)	Strength (%)
Spirits (*brännvin*)	4 73	about 40
Other strong spirit	1 15	26-55
Strong wine	1 17	18-26
Light wine	1 90	8-18
Beer strong (Class III)	1 16	max 4 5
Beer (Class II)	2	– 8
Beer weak (Class I)	10	– 1.8

In order to prevent the abuse of alcohol, a law was passed in 1913 and revised that year and again in 1951 According to this law local committees for the prevention of alcoholism and for the treatment of alcoholics and in every district a county temperance board should study the consumption and abuse of alcohol in the district These committees are supervised by the Govern

ment (National Social Welfare Board of Sweden)

Sale f all alcohol is also in the hands of a State Company (*Nya System AB*) This company sells all drinks stronger than beer class II The sale is controlled by the Control Board (*Kontrollstyrelsen*) and is responsible to the Department of Finance.

DEFINITIONS OF ABUSE OF ALCOHOL AND ALCOHOLISM

WHO suggests the following definitions to define the terms "excessive drinking" and "alcoholism"

Excessive drinking is any form of drinking which in its extent goes beyond the traditional and customary "dietary" use, or the ordinary compliance with the social drinking customs of the whole community concerned, irrespective of the etiological factors leading to such behaviour and irrespective also of the extent to which such etiological factors are dependent upon heredity constitution, or acquired physiopathological and metabolic influences.

Alcoholics are those excessive drinkers whose dependence upon alcohol has attained such degree that it shows noticeable mental disturbance or an interference with their bodily or mental health, their inter-personal relations, and their smooth social and economic functioning; or who show the prodromal signs of such developments. They therefore require treatment.

In his monograph "The Disease Concept of Alcoholism" JELLINEK 1960 showed that the term alcoholism is defined in different ways and that the concept of the term varies widely He found this to be natural since the habits in different countries differ so widely and he suggested the use of the following definition. Alcoholism is any use of alcoholic beverages that causes any damage t the individual r society r both.

This definition is rather ague and JELLI EK theref re gives definitions of five species f alcoholism

In Sweden the terms abuse of alcohol and alcoholism are common.

In the investigation of *Nykterhetsnämnden* the term abuse of alcohol is defined ecording to the data available at the authorities watching over the use and abuse of alcohol and is divided into 3 groups Group 1 includes those who have been taken care of by the com munity according to § 1 of the law of abuse of alcohol during the years 1943—1945 To Group 2 are assigned those who have had at least 3 convictions for drunkenness o for other misbehaviour under the influence of alcohol during the years 1936—1945 and Group 3 includes persons who, on some occasion or an ther were reprimanded by th temperance board in 1936—1945

ABUSE OF ALCOHOL IN SWEDEN

Of the men above 15 years, Group 1 consisted of 0.57 %, Group 2 of 1.32 % and Group 3 of 5.82 %, *i.e.*, total of 7 7 %. In the towns, the total of the 3 groups was 10.6 % For the women in the whole f Sweden, it was 0.17 % and in the towns 0.32 %.

ESSEN MÖLLER et al. (1956), who studied a provincial community found that 13.5 % of the adult population abused alcohol or were still abusing it. According to ESSEN MÖLLER (per sonal communication) the same def inition was used as KAIJ s (1960) in his account, *i.e.*, abusers are heavy consumers and chronic alcoholism is characterized by 1) a pathological desire for alcohol after ingestion f small quantities, 2) regular black-outs during intoxication with alcohol, and 3) a physical dependence on alcohol. KAIJ stressed the difficulty in distinguishing alcohol abuse from normal drinking and therefore divided his material into 5 groups according to drinking habits, as noted in the registers of the temperance board.

The number of persons who had been convicted for drunkenness in 1954 because of drunkenness in Sweden, and which is usually taken as a measure of the abuse f alcohol was 7.8 per thousand

inhabitants 15—70 years of age This figure rose in 1956 to 16 1 and in 1959 it was 13 1 It should be observed that the data refer to the number of offences and not to the number of individuals According to Åmark (1959) a large number of offences were referable to a single person The number of offences was greater in the towns than in the country For Västerås, the figures were between the average for the town and country *i.e.*, 8 0 *offences per* 1000 inhabitants in 1959 (the question of alcohol in Västerås White book 1961)

In ESSEN MÖLLER's series from the country only one third of those classified as abusers were known to the temperance board. KAIJ (1960) therefore studied the representativeness of the clientele of the temperance board and found that it could be regarded as a random sample of all abusers from a social and medical point of view

THE PROBLEM OF ALCOHOL IN INDUSTRY

What alcohol really means to industry is only partly known. Such terms as "the concealed problem" (HENDERSON & BACON) and "the concealed man and the concealed costs" have been coined

Several attempts have been made to assess the number of alcohol abusers in industry Even in such investigations the result will also vary with the definition. According to Yale Center (cit MORGAN 1958) about 80 % of the Company's servants use alcohol Of these, 6 % are problem drinkers and of these *one* fifth may be regarded as alcoholics TRICE (1959) reported that the mean number of alcoholics in USA's industry was about 3 % with a range of 0—10 % Data for Sweden are rare *LINDGREN* (1957) *found* that among 3000 men, 199 had been reprimanded because of drunkenness during work. FORSSMAN (1959) is of the opinion that 4—5 % of all employees *have alcohol problems*

Correlation between alcohol consumption and sick absence

According to JELLINEK (1941) who is often referred to problem drinkers are on the average absent 22 days more per year than others Two of these days should be regarded as sick absence and the rest as voluntary absenteeism in association with debauches These data are taken from STEVENSON's investigation (1940) on people working in a mine and they were not checked again until *the end of the* 1950*ies*

LINDGREN (1957) *showed that absence because of illness including accidents* among 3 000 outdoor workers during the years 1951—1956 was fairly constant and about one month per year (28—31 9 days/year) Among *199* alcohol abusers (*i.e against whom disciplinary measures* had been taken because of drunkenness) absence varied in the same year between 33.5—51.5 days LINDGREN was able to show that the variation is dependent on sick absence and he found that the *difference* between *abusers* and the remainder was not due to short repeated absences The distribution among abusers of the diseases in the diagnostic groups was the same as that of the remainder but the *abusers had an overmorbidity* particularly regarding the diseases of the organs of locomotion but also in diseases of the respiratory tract mental diseases and accidents particularly accidents *outside* working hours

MAXWELL (1959) who studied a company in the USA with about 10 000 employees, found 48 to be problem drinkers (32 males 16 *females*) *but it* was pointed out that these were certainly not all the problem drinkers and that it was not with certainty a random sample These 48 were however compared with 2 control groups matched for *age sex occupation and duration of service* in *the* Company Absence of more than 8 days was studied for the entire period

of employment which in the problem group was found t be 27 years, on the verage, and in th control group 28.5 years. The average sick absence for the problem group was 213.2 days against 96.8 days for the controls. Among the men, sick absence was 2.9 times as long in the problem group Th number of absences also showed the same tendency 7.6 for the problem group against 3 1 for the controls. THORPE et al. (1959) gave similar results for problem drinkers who represented 0 71 % of the 37,000 mployees

Absence because f accidents has received special attention. Theoreti cally the risk of accidents hould be greater during acute intoxication, even of moderate degree, since even small doses of alcohol are enough t lower working capacity (GOLDBERG 1943 1947 DREW 1961)

It has been found that the risk of accidents is directly related to the alcohol content of the blood (LUCAS et al. 1955) POPHAM (1955) however expressed th view that the increased risk applies t all abusers, independently of h w much they might hav drunk at the time of th accident. MAXWELL (1959) found that accidents among workers over 40 years did not differ from the control group Below 40 years, however the risk was twice as high fo the problem group, particularly accidents outside working hours where the ratio was 21:0 If accidents during work be included, the ratio would be 3.6 1

VARIABLES

T elucidat the consumption of alcohol f the proband nine variables were studied (Fig 41 and Table 32)

1 2 The number f off nces f drunk enness from 1920 until 31.12.1959 and the number f years si c last off nce

D t on drunkenness were obtained from the registers of the temperance board in Vä terås (NN) There all offences because of intoxication since 1920 are registered, even if the inhabitants had been intoxicated elsewhere in Sweden One might imagine that those who moved into the town relatively lat would be under-represented in the registers of the temperance board. To check this point, 50 consecutive cases in the material were studied also in the registers of the control authorities which cover the whole country Complete greement was found between the two registers except fo the very latest offences, which had been too late to be entered into the registers of the control authorities

The majority f the cases have been reported to the temperance bo rd after the probands had been arrested by the police because of being drunk and disorderly or they had been unable to take care of themselves. Only rarely are cases of intoxication at home reported to the temperance board, and then via relatives neighbours.

It was considered of value t the investigation to ascertain the scope of the term drunkenness. The author therefore interviewed a police sergeant in Västerås, who had had 20 years experience with people arrested because of drunkenness. He reported that it was always the sergeant on duty who decided whether or not person brought to the police station was t be considered intoxicated. He reported that it was usually easy t decide whether a man was intoxicated or not, although it was sometimes difficult to distinguish it from some other type of intoxication. In doubtful cases they consulted a doctor *Blood* specimens were drawn only of persons arrested fo drunken driving. The police have no definit instructions to go by when deciding whether a person should be regarded as intoxicated or not: in other words, they must rely on their own experience with su h cases

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3 *Drinking habits.* (Fig 41)

The probands were classified according to alcohol consumption in groups a—e.

) teetotalers

b) consumption of alcohol without delict

c) consumption of alcohol with delicts according to the temperance board but n t known to medical depart ment as abusers or alcoholics

d) alcohol consumption with delict according t the temperance board and known to the medical department as abuser or alcoholic

) consumption of alcohol without delict according to the temperance board but known to the medical depart ment as abuser or alcoholic

The evaluation is based on a personal interview conducted by the author data from the registers of the temperance board and medical department of ASEA.

Only those who on principle or for ther reasons had always been teetotalers as judged by the norms of the abstainer associations belong to a). According t these norms, the alcohol content of a drink hould not exceed 1.8 ₒ/volume Present membership f an abstainers association is thus not eno gh. The word of the probands was relied upon.

Persons who had been drunk at their w rk, who had been reported by the f remen because of drunkenness or known t the Social Office f the Company that they were drunkards as well as those who had sought advice at the medical department f r their alcohol problem were registered as busers or alcoholics

In the present investigation there was no reason t draw line between abusers and lcoholics because there were so few alcoholics.

4—6 *Reported consumption of spirits in liters n 1959 Trend f consumption of spirits since 1955 Number of years of spirit consumption before 31 12.1959*

Variable 5 is clasified in groups a—c.

a) *Increased consumption*

b) unchanged consumption

c) decreased consumption

It might appear difficult to obtain reliable inf rmation on such sensitive question as the consumption of spirits at an interview and, in addition, 1—5 months after the time in question However the author has the impression that the probands did their best to give correct information. The interview was started by discussion of the time when the sale of spirits in Sweden was rationed, when it was known how much each individual purchased. The next point was to find out whether the amount of alcohol consumed had since increased or decreased. If they reported substantial changes in the consumption of alcohol, they were asked what induced them t do so It is obvious that not all f the answers were absolutely correct, but the author feels that the error due to incorrect information can be regarded as negligible, at least for the teetotalers and those who had drunk only a moderate mount of alcohol without delicts.

7—9 *Number of tots of spirits number of glasses f wine and number of bottles f beer per week.*

At the interview the proband was requested to make a n te of what he eat and drank during the following week, and to return the form which he received duly filled out t the author If they did not return the form, they were reminded to do so by letter If they still failed to return the form, they were not reminded or requested again. With this procedure there appears hardly to be any reason

With the increased demands on public security one might imagine that the police have become stricter in their judgement of what should be regarded as drunken driving

At any rate, opinions differ widely as to what should be considered drunkenness ALLARDT (1957) for example, found different concepts of the terms abuse and alcoholism among different groups of the population classified according to their own consumption of alcohol. teetotalers apply the word in a much wider sense than people with a moderate consumption. The percentage of teetotalers among the population varies widely from one district to another in the country (29 % in Jönköping district and 5 % in large towns) so that the opinion of what should be understood as drunkenness certainly varies also Thus LILJESTRAND (1940) showed great differences in the evaluation of the degree of drunkenness, as judged by different doctors

Drunken driving is a severe offence and can be punished if the blood content of alcohol is 0.05 % or more This is of less importance in the present material because it included only 2 cases of intoxication at the wheel and those 2 persons had also been arrested because of drunkenness on other occasions

ESSEN MÖLLER et al. (1956) as mentioned, found only one third of alcohol abusers to be known to NN This does not probably apply to the present material, because all offences since 1970 have been registered. Only 4 alcoholic abusers known to the Company were not known to the temperance board On the other hand the records of the temperance board contained the names of a number of persons who at any rate at present, are not regarded as abusers. one has been a teetotaller for 10 years, and most of them had their last conflict with the police more than 10 years ago

The terms abuse and offences of drunkenness are thus not synonyms but drunkenness is better defined because all offences are recorded.

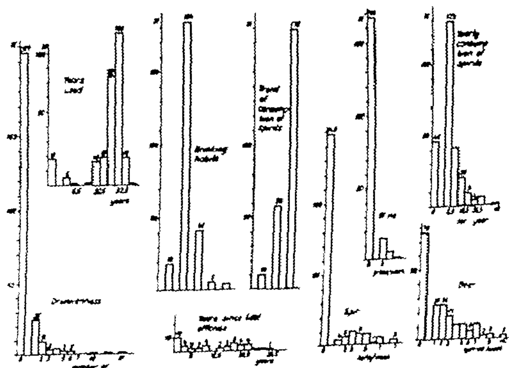


Fig 41 *Distribution of probands according to alcoholic variables*

Table 33. *Tobacco consumption (Means and standard deviations)*

	N̄	M̄	S
Cigarettes/day	252	3.0	4.9
Cigars/week	253	0.3	2.5
Tobacco (gm.)/week	252	16.6	21.2
Years smoked	252	21 4	12.6
If stopped, when last smoke	42	11.5	7 7

CONSUMPTION OF DRUGS

It is natural that sick people consume more drugs than healthy But it should also be borne in mind that healthy people consume a fair mount of drugs although they are not indicated on medical grounds. These drugs may Som times cause injury Hence, it was considered necessary roughly t assess the consum ption of certain drugs.

Consumption f analgetics sedat ves and narcotics

Table 34.

		N
grade	1	203
	2	41
	3	7

A 3-grade scale was constructed

1 Consumption seldom or never

2 Consumption on special occasions in association with diseases of hort du ration

3 Fairly regular consumption

It is obvious that one cannot expect narcomaniac to report his consumption at an oral interview with a doctor but since the uthor had access to the records of the medical department and was well acquainted with the foremen and the working environments as well as the workers, he feels that the material incl ded no narcomania The author is also of the opinion that the consumption reported was medically justified. Number in this variable, 251

WORKING CONDITIONS

The work done in modern fact ry covers wide range f jobs so that it was necessary to construct different systems for the evaluation of different types of w rk from different points of iew When engaging people r transferring them from one job to another the capacity f the person must be j dged in relation t the requirement of the work, in order to get the right man at the right place In ind try some types of work can be done by persons who do not enjoy really good health. For this pur pose in 1919 ASEA introduced a system designed by HANMAN HANMAN calculates th time in hours d ring w rk day required by different and defined f ctors. In special occupation, for example the work may require the man t lift 50 kg in one hou it m y be necessary t work indoors for 8 hours it may be necessary to carry articles weighing less than 10 kg for 8 hours, it may be necessary to walk or sit r manipulate different things, etc. fo a gi en number f hours. The sy tem ha the disadvantage that the analysis soon becomes out-of-date owing to the present rapid rationalization of industry It has also been claimed that the maximum values given are too high. HAN MAN analysis is till of value when engaging new people, but it does not giv sufficient information for the pur poses of the present investigation. It was therefore decided to use another system

to suppose that the men did not give correct information but it might have influenced the consumption of alcohol that week Answers were received from 195 (78 %) of the probands

Table 32. *Variables elucidating use and abuse of alcohol (Means and standard deviations)*

	N	M	S
Number of offences for drunkenness	261	0.5	1.8
If offence for drunkenness years since last *offence*	48	10 8 yr	8 4 yr
Drinking habits	25	See Fig. 41	
Reported consumption of spirits	251	4 6 L	6.6
Development of consumption	247	See Fig 41	
Years alcohol used	250	25 8 yr	8.3 y
Bottles beer/week	195	3	2.7
Glasses spirits/week	195	See Fig. 41	
Glasses wine/week	195	See Fig. 41	

CONSUMPTION OF TOBACCO

Injurious effect of tobacco on the health is at present receiving world wide interest and a large number of investigations have already been published. Suffice is here to refer to PROOSDIJ's monograph (1960) which contains discussion of a large number of investigations

It is obvious that in an investigation of this type the consumption of tobacco cannot be ignored First of all, it is important to have an opinion of the consumption of tobacco among industrial workers in Sweden and, secondly to find out whether the consumption of tobacco has anything to do with sick absence

It should be observed that since 1949 smoking is allowed in certain workshops of ASEA. Formerly all smoking in workshops was forbidden but the time lost on the way to and from places where smoking was allowed proved too great In those workshops where there is no risk of fire, smoking is now allowed In some workshops smoking is still forbidden It is obvious that the consumption of tobacco is influenced by these regulations

It was not possible to study the smoking habits in detail i.e., whether they inhaled the smoke what sorts of tobacco were used, whether they smoked during the day and whether they smoked the cigarette to the very end or threw away large stumps The interview of smoking was limited to the following details The results are given in Fig 42 and Table 33 The following variables were used

Numbers of cigarettes per day numbers of cigars or cheroots per week. Grams of pipe tobacco per week number of years of smoking before 31.12.59 and if stopped smoking how long ago?

The consumption of snuff and chewing tobacco proved so little that it could be ignored

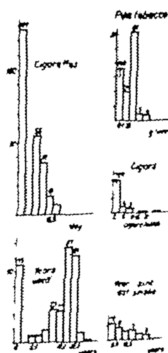


Fig. 42. *Distribution of probands according to smoking habits.*

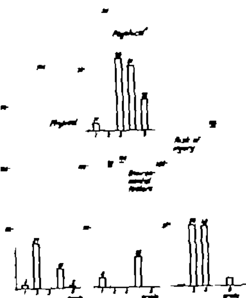


Fig. 45. *Distribution of probands according to demands on stamina and other conditions of working place*

As mentioned, the data were obtained from the registers. In addition, however the working places were visited t check the justification of th merit system. In a few ases, typing errors had occurred and were corrected in association with the foremen.

The values noted do not cover the scope of all that is t be understood by the names f the factors. This was particularly b ious regarding psychical tamina. This variable refers only t monotony of the w rk perf rmed by people who ha e t sit or stand t the same place the whole day The name of thi factor is less suitable but is deeply rooted and will theref re be used here

The following ariables were also studied at the working place

Heaviness of work (Fig 47) This variable gives the a erage amount of energy required by the work per day The variable is not identical with physical exertion Measurements made at ASEA's workshops in 1955 by Doc. N LUNDGREN could be used. These measurements were used as references in the evaluation. In difficult cases, the pulses of the men were measured during actual work. The material was divided according to the following 5 grades

1 Very light work. Pulse rate less than 75/min.
2 Light work. Pulse rate less than 75—100.
3. Moderately heavy work. Pulse rate less than 100—125
4 Heavy work. Pulse rate less than 125—150
5 Very heavy w rk. Pulse rate less than 150—175

Muscular strength required (Fig 47) The maximum weight to be lifted with ut help during the course of a day's work was j dged together with the workers and f reman. The weight is given in kg. with an accuracy of 10 kg It was ignored whether the weight had to be lifted nce or more often a day If this had been considered, it would have influenced the variables, heaviness and weight and physical exertion.

Demands placed on posture and locomotion (Fig. 4) When preparing the present investigation, it was observed that one man was particularly useful because f the flexibility of his joints H had to creep through pipes and inspect the work and clean generators through very small openings etc It was therefore considered to judge also what demands an occupation places on posture and locomotion.

for evaluation of work which was designed in order to attain a fairer adjustment of wages This system has been used in the USA successfully for a couple of decades (LYTLE 1954) There are 4 main ways of assessing work, rank system, classification system comparison of factors and point system (FKO meddelande No 32 IVA 1959) ASEA s system which is based on American experience and adapted for Swedish conditions was first applied in 1949 and the system as it is now used, was introduced in 1957 It is based on the point system but has been supplemented with factor comparison the point system has been designed for 29 key occupations The definitions of the different factors and the description of assessment of the work are apparent from Appendix

In the present investigation the distribution was carried out in points for treatment in Facit EDB and in grades for the graphical illustration in Figs 43—52

Four Variables on *demands on dexterity* are given in Fig 43 the three variables *demands on sense of responsiblity* in Fig 44 and two *demands on stamina* and *other conditions at working place* are collected in Fig 45

The value for the occupation the proband had in 1959 is given If he had not worked in 1959 because of illness, the value given is that for the occupation he had before he fell ill.

Occupational merit factors given in Fig 46

The system for assessing the work of a proband is supplemented by a merit system The definitions of the various factors and their use are given in Appendix Each worker is given a value for his merits every year and the values are noted at the personnel office In the present investigation the mean number of points for the 4 years 1956—1959 were used

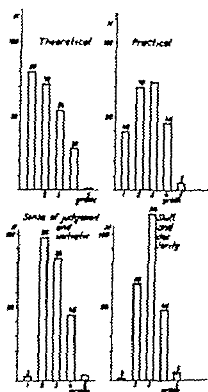


Fig 43. *Distribution of probands according to demands on dexterity*

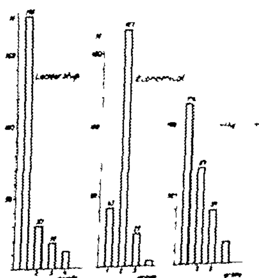


Fig 44 *Distribution of probands according to demands on sense of responsibility*

conversation with fellow w rkers and foremen had any effect on sick absence.

A 3-grade scale was constructed in the following way

1 W rking togeth r with others in a room r factory where conversation was possible with ut leaving the working place or raising voice and wo k necessitating conversation with thers

2 Normal factory environments in which one must leav on s work to talk to others or raise one voice t be heard. Communication hardly necessary at work.

3 Working in isolated environments without contact with thers, e.g crane dri ers, or people working in such noisy environments that con versation is impossibl

Average noise and maximum noise (Fig 48)

The noise was measured at stationary w rk places and for those working in different parts of the factory by an engineer (B Olsson) N attempts were made t measure the noise outdoors or the noise to which mot r drivers are exposed. It was originally intended t mak the measurements with an ppa ratus designed f the octave band filter This, however pro ed impossible because of lack of time since the use f the octave band filter would have required several day measurement at every working place for high frequency noises occurred sometimes when the apparatus wa djusted for low frequency and vice versa. Thi is because noise from surrounding workshops was irregular and could not be predicted. It was therefore necessary t limit the investigation to the use of the general noise with the aid of a General Radio Sound Level Meter Registrations were made f the noise at the working place and of maximum levels in the course of a day

Measurement were made with an



Fig. 48. Distribution of probands according to noise at place of work

accuracy of 1 dB It should be pointed out that measurements were made on one occasion only There was no possibility of judging changes in the noise in the course f the year It was therefore not considered necessary t make measurement more ccuratelv than what was done

Work ndoors and utdoors (Fig 49) Time in hours for indoo work was noted. Indoor work is t be understood as work in workshops with t mperature of at least 1 C.

Shift work

a. Ordinary from 7.00 to 17.00 hours with break between 11.30 and 13.00 Mondays and Fridays, Saturdays 7.00 to 1..42 with 12 min. break.

b Two-shift Mondays to Fridays 5.00 11.00 break 8.30 to 9.00 or 14.00 to 23.00 and break 18.30 to 19.00 Saturdays 5.00 t 10.30 or19.30 to 16.00.

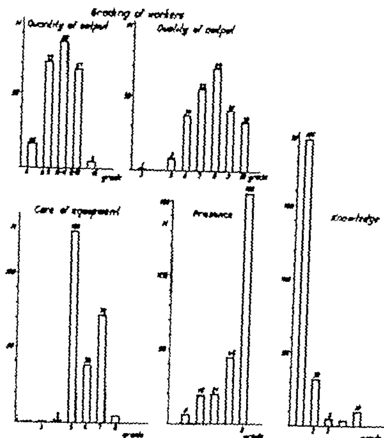


Fig 46 *Distribution of probands according to merit*

A 3-grade variable was therefore designed.

1 Work in comfortable posture, mainly sedentary with a possibility of changing posture

2 Ordinary factory work during which the worker is standing or walking the whole day

3 Work in less comfortable posture or placing high demands on flexibility

Speech interference at place of work (Fig 48) This variable is probably related to "psychical" stress but the purpose was to find out to what extent

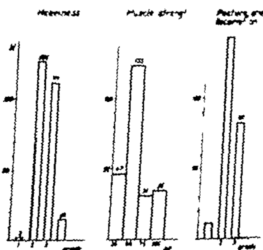


Fig 4 *Distribution of probands according to other variables of requirements of work*

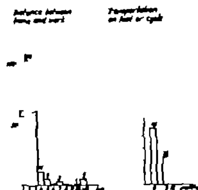


Fig 51. *Distribution of probands according to distance and mode of transportation to work*

by car train or bus, are given in number of month per yea

These dat were given by the proband. Th method f transport varied, of course with the distance The method of transport also varied with the season and health, ju t a the method of transport may ha influenced health The number of month per yea by the different methods of transport were used.

The total of the 3 variables hould be 1_

Influence of the conditions at the working place on health also ary with the time exposition

The following variables were also studied

Duration f employment at ASEA (Fig 52) The time i given in number f yers and calcul ted t 31 1_59

Tim at present job If the probands ha had the same occupation before they entered ASEA, the number f years was added. If they had had another occupation, only the number of years for the present occupation was consi dered

Number of different job (Fig. 52) If the proband had had the same job in different companies or work in different departments in the same company the jobs are counted as separat jobs The changes which occurred now and then at the same working place owing to ra tionalization, were not counted as sepa rate jobs.

Table 35 *Variables illus rating working conditions etc (Number studied means and standard deviations)*

	N	M	S
Demands of work ent			
Theoretical knowledge	236	1 4 poin	1.4
Practica kill	236	4.9	2.0
Sense of judgement and initiativ	236	5.4	_4
Skill and dexterity	236	5.8	_2
Sense of responsibility for:	236		
others safety	236	1.0	1.5
Company's expenses	236	3 0	1.6
leadership	236	1.3	2.4
Stamina			
Physical	236	5.	2.1
Psychical	236	2.0	1.1
Other factors			
Environmental	236	5.1	2._
Risk of injury	236	_6	1.2
Merits			
Quantity	234	10.3	1.8
Quality	234	.8	1.4
Equipment and mat rial	_34	5.	1.0
Presence	234	7.3	1.2
Knowledge	234	1 1	0.9
Heaviness of work	234	_5	0.6
Muscle strength	236	46.0 kg	25.0
Demand on bones and joints	236	2.3	0 7
Conversation possible	234	2.1	0.6
Indoor ork	236	7.0 hr	2.4
Shift ork	236	See Fig. 49	
A erage hour money	231	5_4 Cr	0.57 Cr
P yment system		See Fig. 50	
N ise, erag lev l	21	80 dB	8.8
Noise maximum level	217	86 dB	10.0
Distance home- ork	232	_5 km.	_4
Walking or cycle	231	8.8 mth.	4._
Autocycle or motorcycle	231	1.3 mth.	2.1
Car bus, train	231	2.0 mth.	3.6
Employment in Company	234	_1.5 yr	8.9
Time at present job	232	14.4 yr	10.0
Number of different job	23	2.9	1.6
Change of job because of health	23_	88 (yes)	192 (no)

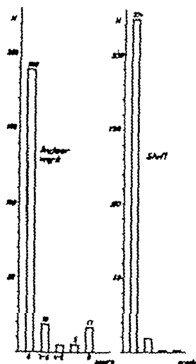


Fig. 49 *Distribution of probands according to type of work.*

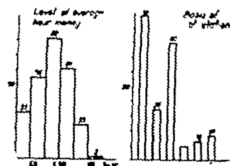


Fig. 50. *Distribution of probands according to earnings*

c Three-shifts Working hours Fridays to Mondays varying between 6 00 and 14 00 14 00 to 22 00 22 00 and 6 00 Saturdays 6 00 to 14 00 and 22 00 to 6 00 Varying breaks

d Night work. Working every other night from 17 00 to 7 00 with varying breaks Continuous work did not occur in the material

Average wage per hour (Fig 50) was calculated every year because holiday money is based on this amount The average for the years 1956–1959 was noted in number of *öre* per hour

Basis of calculation (Fig 50) Wages of the men in the group studied were based on the following calculation principles

a) Piece work. Wage proportional to performance

b) c) Mixed piece work Wage in form of a fixed and a variable portion The variable portion depend on performance

b) direct mixed accord The variable part depends on the performance of the individual

c) Indirect mixed piece work The variable part depends on the work of the group or department

d) Team piece work. Team accord may be paid as a pure piece work or some form of mixed piece work

e) Time wage hour A time work i given to those for whom piece work i not available The wages are decided according to the nature of the work Workers with good merits may be given a supplement

f) Weekly wages Some workers are guaranted a weekly wage They may also be on direct or indirect piece work. If so, in the present investigation they were placed under b) or)

At the border between condition at the working place and at home th following variables were tudied

Distance from home to working place A given in km (Fig 51)

Method of tran port by foot or by cycl (Fig 51)

by autocycle or motorcycle

by 4 factors Skeletal length factor sturdiness factor muscle factor and fat factor His system gi es relative values

It is bove all, the amount f f t that has attracted interest, and many different methods have been devised for evaluating thi component. If only stature and body weight be considered, the error of the method will, according to v DÖBELN (1960) be 10 %. The most reliable method is hydrost tic weighing but this method is time-consuming and incon enient. v DÖBELN (1959) therefore recommended a formula f r calculating the amount of fat free weight from tature, the radio-ulnar breadth and femoral condylar breadth. It is also possible to assess the relative amount of body fat by measuring skinfolds Different measuring pparatuses have been dev sed, but they are often dependent on the examiner so that it is difficult to obtain comparable values. EDWARDS et al. (1955) analysed th methods critically and described an apparatus and a standardized method for btaining comparable alues.

LINDEGÅRD 4 factors have pro ed ery val able n rious inve stigation LI DEGÅRD & NYMAN (1956) demonstrated the correlat on between body-build and personalit type described with SJ BRING s radical LINDEGÅRD & FORSSMAN revealed a correlation between bod -build and coronary sclerosis, nd BJURULF (1959), between body build nd atherosclerosis

VARIABLES

In mea ring bod build the following ariables were used. Measurement were made by nurse Göt Andersson.

He ght (See Fig. 53). The subjects were measured w thout support in standard erect position ad mod in MARTIN (1928) N object with any spinal deformity or deformity f the lowe limbs influencing body height were included. Mea urement were made between 9 a.m and 11.30 a.m by the same examiner throughout. The subjects began work at 7 a.m. and had thus been up since about 6 a.m. Diurnal variation of stature had thus, as far as possible, been eliminated.

Body weight (Fig 53) Body weight was measured after voiding of the urinary bladder and t the same time as above .e 2.5–5 hours after breakfast. No allowance was made for the intestinal contents. The men were weighed with an accuracy of 0.5 kg

Length factor (Fig 53) The lengths f th tibia and of the radius were measured according to the method of LINDEGÅRD (1956) to nearest 0.5 cm For calculation of the lengthfacto th measurements n ted were fed into Facit EDB where they were converted from length in cm to SD-units, and added.

Sturd ness factor (Fig 53) LINDEGÅRD s (1956) method was used. The alues found for the femoral condylar breadth and bimalleolar breadth were con erted to SD-units and added. The measurements were made with an accuracy of 1 mm

Muscle factor (Fig 53) By means of a specially designed dynamometer according to STOLTZ & STOLTZ (1951) muscle strength of the left hand and of the right were measured as well s f the houlder pull and shoulder thrust. All 4 measurements were made 3 times The highest value n ted f r each f the mea urements wa accepted The three ariables were con rted int SD-units and dded In ddition, use was made of a dynamomet r recommended by LUNDGREN f r measuring the strength of the muscles of the back. The dynamometer is described by HANSSON (1961) It was found that the result was dependent t high degree on back pain and to l fting technique This measurement was therefore not used in the evaluation f the muscle fact

Fat factor (Fig 53) The amount of body f t was calculated in 4 different ways

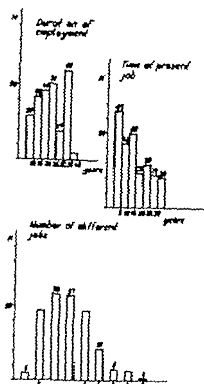


Fig 52. *Distribution of probands according to duration of service in company and variation of jobs*

Information on the duration of employment and number of different job they had had was given by *the proband* themselves for the time before they had joined the ASEA and from the work cards for the time they had been with ASEA

Was the choice of present job influenced by state of health? Change in occupation or job may occur at the request of the foreman of the worker or the doctor

Whether a person had changed his job because of health was decided by the author on the grounds of data given by the proband and of data from the medical department

STATUS

BODY BUILD

HISTORICAL

Even HIPPOCRATES thought that health was to some extent dependent on body build, as is apparent from his terms habitus phtisicus" and habitus apoplecticus KRETSCHMER has demonstrated the correlation between 3 extreme types of body build and endogenous psychoses He described body build mainly verbally Since then various systems have been devised for objective measurement The simplest is the relationship between stature and body weight, which is used, above all, for judging the amount of body fat The relationship may be expressed in two ways either as centimetre-weight (weight in kg — body height in cm — 100) or a ROHRER s index

$$\frac{\text{Body weight in kg} \times 100}{\text{Body height in cm}}$$

Tables for normal weight for each sex and for age have also been published These methods have been used for example in American Insurance Statistics and it has been shown among other things that over weight is associated with over mortality (MARKS 1956)

It has however also been found that body weight is correlated not only with the stature and amount of body fat but also with the sturdiness of the skeleton In 1937 STRÖMGREN introduced an index for assessing the cross-sectional area of the chest in relation to stature In 1953 LINDEGÅRD showed that the musculature must also be considered According to LINDEGÅRD body build can be described

by 4 factors: Skeletal length fact turdiness facto muscle factor and fat factor His system gives relative values.

It is, above all, the amount of fat that has attracted interest and many different method have been devised for evaluating this component. If only stature and body weight be considered, the error of the method will, according t v DÖBELN (1960), be 10 The most reliable method is hydrostatic weighing, but this method is time-consuming and incon enient v DÖBELN (1959) therefore recommended a formula for calcula ting the amount of fat free weight from stature the radio-ulnar breadth and femoral condylar breadth. It is also possible t assess the relative amount f body f t by measuring kinfolds Differ ent measuring apparatuses have been devised, but they are ften dependent on the ex miner so that it is difficult to btain comparable values EDWARDS et al. (1955) analysed the methods critically and described an apparatus nd tandardized method f r obtaining comparable val es

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VARIABLES

In measuring bod -build the f llowing ariables were used. Mea urement were made by n rse Göta Andersson

H ght (See Fig 53) The subject were measured w thout support in tandard erect position *ad modum* MARTIN (1928) N subject w th any pin l deformity r d formity of the lower limbs influencing body height were included Mea urement were made between 9 a.m. and 11.30 a.m. by the same examiner throughout. The subjects began work at 7 a.m. and had thus been up since about 6 a.m. Diurnal variation of tature had thus, as far as possible been eliminated.

Body weight (Fig 53). Body weight was measured after voiding of the urinary bladder and at the same time as bove i.e 2.5—5 hours after break fast. No allowance was made f r the intestinal contents. The men were weighed with an accuracy of 0.5 kg

Length factor (Fig. 53) The lengths of the tibia and f the radius were measured according to the method of LINDEGÅRD (1956) t nearest 0.5 cm. For calculation of the lengthfactor the measurements noted were fed into F ct EDB where they were converted from length in cm to *SD-units, and added.*

Sturdiness factor (Fig 53) LINDEGÅRD's (1956) method was used The values found f r th femoral condylar breadth and bimalleolar breadth were converted to SD-units and added. The measurements were made with an accuracy of 1 mm.

Muscle factor (Fig 53) By means of a specially designed dynamometer according t STOLTZ & STOLTZ (1951) muscle strength of the left hand and of the right were measured as w ll as of the houlder pull and houlder thrust All 4 measurements were made 3 times. The highest value noted f r each of the mea urements was accepted The three variables were con erted int SD-unit and added In addition, use was made of a dynamometer recommended by LUNDGREN for mea uring the strength of the muscles of the back. The dynamometer is described by HAN SON (1961). It was found that the res lt was dependent to a high degree on back pain and to lifting technique This measurement was therefore not used in the evaluation of the muscle factor

Fat facto (Fig 53) The amount of body fat was calculated in 4 different w y

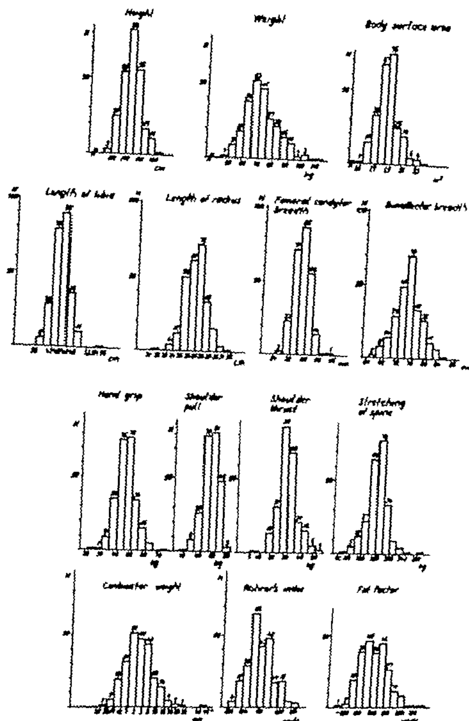


Fig 53 *Distribution of probands according to body build*

1 Centimeter weight (See page 100)

2 Rohrer s index (See page 100)

3 Fat factor according to LINDEGÅRD The factor is calculated from the body weight after elimination of the effect of height sturdiness and muscle factors It was not possible to use the *formula of* LINDEGÅRD (1953) for calculation of the relative fat

factor because the formula requires a normal distribution of the different variables. Therefore the following difference was calculated, instead.

weight — x(length + sturdiness + muscle)

The difference found shows whether the fat factor of a given person is above or below the statistical average, expressed in SD-units.

Skinfold measurement (Fig 54) Skinfolds were measured at 3 sites on the body For technical reasons the measurements were made on the left half of the body

1. On the back just below apex scapulae dinster
2. On the chest, at the lateral edge of the major pectoral muscle in the anterio axillary line.
3. On the abdomen, about 5 cm. t the left of the umbilicus.

All skinf ld measurements were made by the author

In most previous investigations of this type in Sweden use has been made of an apparatus designed by KEYS In the Company department for measuring instruments this pparatus was, however for several reasons considered unsatisfactory and another apparatus was constructed which largely resembles that described by EDWARDS et al. in 1955 Certain diff rences should, how ever be mentioned. The faces of the callipers were 5×20 mm. instead of 6×15 mm. The pressure between the shanks was 3 g./mm.² instead of 9—20 g./mm.² Despite these deviations from the norms recommended by EDWARDS, the apparatus used proved satisfactory on comparison within a material studied by one and the same examiner, as is apparent from the trials performed before the investigation was started.

HEART VOLUME

The heart volume was measured by means of thoroscopy according to the method of LINDGREN & ODÉN (1954) (see Fig 55) (The calculations were made by Dr R. Raxell.)

Since the measurements are made directly on the microfilms in mm. it is necessary to have a correction factor to obtain the true volume in cm³ To btain this constant, some subjects were referred t the Central Hospital in Västerås for t the Central Hospital in Västerås Roentgen department for exammation also with a full-scale film. The value obtained proved to be 1 1 Since relative values were sufficient for the present investigation it was not considered

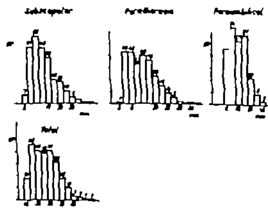


Fig 54. *Distribution of probands according to skinfold thickness.*

necessary to correct for this difference because all of the men were examined by the same method and the same apparatus Moreover all the examinations were performed by the same examiner who checked that the position of the body in the roentgen apparatus was correct.

Body surface area was calculated with an accuracy of 0 1 m² from body height and body weight (Fig 53) with the aid of nomograms by DU BOIS

The relative heart volume was calculated from the formula

$$\frac{\text{absolute heart volume}}{\text{body surface area}}$$

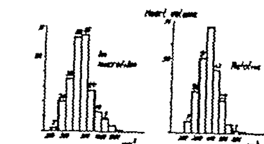


Fig. 55 *Distribution of probands according to heart volume.*

(See Fig 55) Here too, use was made of the uncorrected value found for the heart volume

Table 36 *Body-build factors and heart volume factors. Mean values and standard deviations.*

	N	M	
Body height	251	1 3 cm	6.. cm
Body weight	.5	.8 kg	11.3 kg
Length factor			
Radius length	.52	7 0 cm	1.6 cm
Tibia length	.52	45.3 cm	.. cm
Sturdiness factor			
Femoral condylar breadth	252	9 8 cm	0.4 cm
Bimalleolar breadth	.51	7 5 cm	0.4 cm
Muscle factor			
Dynamometer best hand (handgrip)	250	46.. kg	6 6 kg
" shoulder pull	44	30 7	6.8
" shoulder thrust	46	51..	10.6
" tretching of back	.3	15..7	8.9
Fat factor			
Centimeter-weight	251	See Fig 53	
Rohrer index	.51	See Fig. 53	
Fat facto according to Lindegård	44	See Fig 53	
Skin fold measurements			
Subscapular	.5	14 mm	6.6 mm
Parathoracic	.5	13.6	0
Paraumbilical	.5	18.0	8.4
Heart volume measured on microfilms uncorrected	.39	69	16.6
Body surface are	18	1 9 m	1 8
Relative heart volume	.39	See Fig. 55	

For correction of heart volume i cm³ the values should be multiplied by 1 1

HAIR GROWTH

According to LINDEGÅRD (1956) and BJURULF (1959) body build is dependent on both hereditary and environmental factors. It is difficult to isolate purely hereditary f ctors in an investigation of the present type, but, as pointed out by both of the above-mentioned authors, use may be made of hairgrowth on the body which is determined mainly by hereditary factors (REYNOLDS, 1951). Since BJURULF howed that hair-growth on the chest is correlated with the number of fat cells per unit of volume it was decided to use this factor as hereditary variable.

Hair growth. (Fig. 56).

The hair growth on the chest was assessed according to a five grade scale described below (LINDEGÅRD 1956)

1 Very slight hairiness over sternum and around the mammillae.

2 Hairiness over sternum and around the mamillae covering an area of about 10 cm²

3 Hairiness over sternum and around the mamillae covering an area more than 10 cm² but without a continuity between the haircovered regions.

4 Continuous hairiness over sternum and around the mamillae but not extending towards umbilicus.

5 Continuous hairiness over sternum and around the mamillae as well as between the sternum and the umbilicus.

BLOOD GROUPS

It has also been shown that the blood group according t the AB0-system, which is purely hereditary is correlated with gastrointestinal diseases (AIRD 19 9) This factor was therefore also included

Blood group according to AB0-system and according to Rh-system (Fig 56)

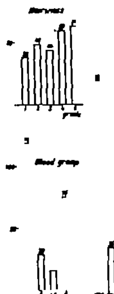


Fig. 56. *Distribution of probands according to heredity*

were determined with the help of Eldon-cards and the technique described in instructions from *Insulinlaboratoriet*, Denmark.

PHYSIOLOGICAL DATA

It is obvious that a series of clinical and physiological data are necessary in an investigation of this type. On the other hand, it is debatable which variables should be chosen. In the present investigation the choice was decided on practical grounds and by the resources vailable at the medical department.

PHYSICAL PERFORMANCE

The probands were tested with the aid of a cycle ergometer ccording to v DÖBELN (19 1) loaded with 300 600 and 900 kpm/min. for 6 minutes each. Bef re the test they were allowed to lie down and rest for 15 minutes, after which the pulse rate and blood pressure were measured and ECG was recorded. During the test the pulse rate and the blood

pressure were measured every other minute The frequency of the pulse was measured with the ECG apparatus If the proband could not complete the test owing to lack of muscular strength or shortness of breath or anginal symptoms the test was stopped. The ECG was recorded with the usual limb leads and chest leads CR 1 CR 2 CR 4 CR 5 and CR 7 During work in the sitting position only the chest leads were recorded. The ECG apparatus used was a 4-channel Mingograph.

The following variables were noted (Fig 57)

Systolic blood pressure during rest.

Pulse frequency during rest

Pulse frequency at 900 kpm /min and steady state

Heart work index at 900 kpm /min and steady state

The heart work index (HWI) was calculated by BUERGER s formula

$$\text{HWI} = \frac{P(S - D + 100)\ (S + D)}{2 \times 10^4}$$

where HWI is the heart work index, P pulse frequency/min. S systolic blood pressure, and D diastolic blood pressure The blood pressure was measured according to the following norms (Apparatus Manotest) The value obtained when the sound appeared on falling pressure was noted as the systolic blood pressure, and the value noted when the sound of the pulse wave disappeared on falling blood pressure in the cuff was noted as the diastolic blood pressure Measurement of the blood pressure during cycling required considerable training In order to prevent contraction of the musculature of the humerus from influencing the blood pressure measured the subject was instructed to remove his hand from the handle bars and allow it to hang relaxed during measurement

CLINICAL DATA

The examination included the usual laboratory studies haemoglobulin,

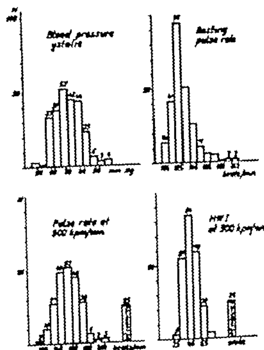


Fig 57 *Distribution of probands according to physiological data.*

E.S R., proteinuria and reducing substance in the urine as well as urine sediment and clinical examination of the heart lung and abdomen the throat, teeth and reflexes When considered indicated by notes in the record cards the examination was extended to include inspection of the ear-drums of the ocular fundi, and rectal palpation

The results of the clinical examination were classified according to the following variables.

Working capacity at time of examination (See Fig 58)

a No working capacity (sick absence certified by doctor)

b Semi-disability Either able to work half a day at usual job or entire day on light work corresponding to about half of the physical requirements of ordinary jobs All of the subjects in this group were registered as semi disabled at the G.S I because of acute or subacute disease

Table 37 *Probands who could not manage 900 kpm/min. on cycle ergometer test*

Case No	Absence 56—59 SAS 311 hours	SAS 310	Cause
25	752	7	Ugly rachitic changes of chest
60	1230	10	Congenital cystic kidney
70	3	1	Muscle weakness
71	1857	21	Morbus Little
76	847	8	Polyarthritis. Cannot cycle
91	48	3	Gonarthrosis. Cannot cycle
94	529	4	Myocardosis. Chronic crural ulcer
101	617	9	Pes excavatus Muscle weakness
105	954	13	Bundle branch block
109	104	3	Pronounced obesity
110	77	2	Atrophy of one leg
119	3800	20	Pulmonary tuberculosis. Muscle weakness
120	3871	19	Postop. status after cerebral tumour
121	128	2	Bone transplant from lower legs. Cannot cycle
135	335	6	Gonarthrosis. Muscle weakness
140	111	2	Chronic polyarthritis
144	1862	7	Acut lumbago. Muscle weakness
147	881	17	Tbc. pulm. with dyspnoea
150	196	2	Gonarthrosis
181	3190	29	Cardiosclerosis
183	1254	17	Bronchial asthma
203	298	11	Ankylosis right knee
224	82	10	Bronchial asthma
235	1144	11	Gonarthrosis

Absence mean 985 hours.

Partial disability To this group were assigned those with permanent loss of working capacity because of some deformity or chronic disease, so that they were only able to do light jobs occupations

d P rmanent p rtial loss f w rking capacity owing to anoxaemia. T this group were assigned those who could not manage 900 kpm /min. t the test, unless they could be classified better under a, b or

N medical reason excl ding them from work in industry f the same type as ASEA.

The di tribution of this variable depends on the hoice f probands tudied i.e random a selection of healthy persons persons who are on the ick list. In the beginning it wa decided t includ a many as possible who were on the sick list, in rder not to cause a loss f too many working hours in the workshops. It was, however soon realized that the investigation would be more valuable if the subjects were examined when they were healthy so that the results of the physical per f rmance test would be as representat ve as possible It wa also found that it was easier t establish good rapport with the ubjects when they were doing their usual work. When possible the subject were theref re given tim to recover from their illness before they were xamined.

This ttempt t examin the workers when they were in health will, of course also influence ergometer recordings and values found for the muscle facto

Evaluat on of the organs f locomotion (Fig 58) The ergometer recordings

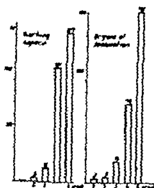


Fig 58 *Distribution of probands according to clinical data.*

(see page 105) are a measure of the capacity of the heart and lungs and registration of the muscle factor a measure of muscular strength But evaluation of the flexibility of the joints of a person and of his capacity to work in an uncomfortable or standing position is also necessary This was judged according to a five-grade scale It is difficult to define the limits to the grades with exactitude Broadly speaking it may be said that those who were only able to perform sedentary work were assigned to group 1 and those who were able to perform any kind of work to group 5

A clearer picture of the various grades may be had from the following examples

1 Polyarthritis or deforming arthrosis in many joints with limited range of mobility and loss of gross functional strength of the arms and legs sequalae after poliomyelitis with atrophy of at least two extremities
2 Joint diseases with moderate loss of range of mobility and of strength weakness requiring the use of a corsette or leather jacket but not preventing work at which the subject must stand or walk for shorter period chronic varicose leg ulcers atrophy in one limb
3 Back weakness on at least one occasion during the last few years ugly varices ugly spinal deformities without subjective symptoms loss of range of movement due to obesity
4 Moderate varices without symptom back weakness in history for 2 years or more Sensitivity to draughts.
5 Healthy good range of mobility no symptoms when standing or working for a long time

Dental status (Fig 59)

SUNDBERG et al (1914) found a correlation between dental status and gastric cancer The teeth were therefore also examined in the present investigation and by the author who had learned the technique from Dr SUNDBERG The technique used was the same as that recommended by The Army Dental *Corps in England in the examination of recruits adding of the number of occlusions of the teeth*

Maximum 26 points which are added in the following way

One point for every incisor and canine in good occlusion

Two points for each premolar in good occlusion

Three points for each molar in good occlusion.

The number of points is reduced if only part of a surface of a tooth is in occlusion. Reduction is also made if a tooth is loose The third molar was excluded if it had assumed the site of the second molar *Complete protheses* were marked with 13

HEARING

Hearing was measured by the audiometrician E JUHLIN The objects were examined in a soundproof room Noise



Fig 59. *Distribution of probands according to dental status*

from outside was not more than 30 dB
Apparatus. Amplivox model 70
The following variables were used to tudy the state f hearing f the workers:

Loss f hearing for speech in dB ccording to the audiogram according to HARRIS et L 1956 (Fig 60)

Loss f hearing was classified according t HEIJBEL, LIDÉN in the following way (Fig 60)

1 Hearing normal. All workers wh on xamination of air-conduction had loss of t most 20 dB f r frequencies of 125 c.p.s —6000 c.p.s.
2. Slight injury du to noise The audiogram normal in the frequency range 125—2000 c.p s but with loss of hearing f air conduction f some f the frequencies 3000, 4000 6000 c.p.s abov 25 dB
3— Severe loss of hearing d t noise To this group were assigned also those workers in whom loss f

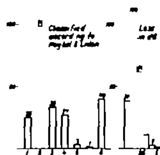


Fig. 60. *Distribution of probands according to impairment of hearing*

hearing was associated with other diseases The audiogram showed a loss of hearing of more than 20 dB lso f one o several frequencies within the peech range 500 1000 and 2000 c.p.s. The loss was graded according to a five grade scale described by HARRIS et al.
8. Other hearing disorders apparently not ascribable to injury by noise

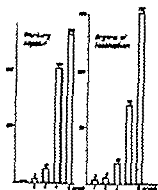
Table 38 *Physiological and clinical data. Means and standard deviations.*

	N	M		
Resting pulse min.	250	71.1		9 1
Resting ystolic blood pressure	250	127	mm Hg	19.6
Pulse at 900 kpm min.	227	153.1		15.5
Heart ork index	227	4.7		1.0
Working capacity t time of examination	23		See Fig 58	
Examination of organs of locomotion	252		See Fig. 58	
Dental st us	232	11.0		5.9
Loss of hearing in dB	223	16.8 dB		8.6
Loss of hearing according t HEIJBEL et al.	223		See Fig 60	

ADJUSTMENT TO WORK

If worker i well adjusted t his job n correlation an be expected between working environments and sick absence In the present in estigation, in which the proband have been employed f at lea t 4 years fairly good adjustment may be ss med. T check this point, however the following variables were t died f correlations.

Heavines f work—pulse frequency t 900 kpm /mm.
Demands placed on muscle strength — shoulder pull, measured by dyn momet r
Demands placed on joints and organs of locomotion — evaluation f joints and skeleton.
The result are given in matrices 3, 4 and 5


Fig. 58 Distribution of probands according to clinical data

(see page 105) are a measure of the capacity of the heart and lungs and registration of the muscle factor a measure of muscular strength But evaluation of the flexibility of the joints of a person and of his capacity to work in an uncomfortable or standing position is also necessary This was judged according to a five-grade scale It is difficult to define the limits to the grades with exactitude Broadly speaking it may be said that those who were only able to perform sedentary work were assigned to group 1 and those who were able to perform any kind of work to group 5

A clearer picture of the various grades may be had from the following examples

1 Polyarthritis or deforming arthrosis in many joints with limited range of mobility and loss of gross functional strength of the arms and legs sequalae after poliomyelitis with atrophy of at least two extremities
2 Joint diseases with moderate loss of range of mobility and of strength weakness requiring the use of a corsette or leather jacket but not preventing work at which the subject must stand or walk for shorter periods, chronic varicose leg ulcers atrophy in one limb
3 Back weakness on at least one occasion during the last few years ugly varices ugly spinal deformities without subjective symptoms loss of range of movement, due to obesity
4 Moderate varices without symptoms back weakness in history for 2 years or more Sensitivity to draughts
5 Healthy good range of mobility no symptoms when standing or working for a long time

Dental status (Fig 59)

SUNDBERG et al (1914) found a correlation betwen dental status and gastric cancer The teeth were therefore also examined in the present investigation and by the author who had learned the technique from Dr SUNDBERG The technique used was the same as that recommended by The Army Dental Corps in England in the examination of recruits *adding of the number of occlusions of the teeth*

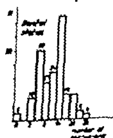
Maximum 26 points which are added in the following way

One point for every incisor and canine in good occlusion
Two points for each premolar in good occlusion
Three points for each molar in good occlusion

The number of points is reduced if only part of a surface of a tooth is in occlusion Reduction is also made if a tooth is loose The third molar was excluded if it had assumed the site of the second molar Complete protheses were marked with 13

HEARING

Hearing was measured by the audiometrician E JUBLIN The subjects were examined in a soundproof room Noise


Fig 59 Distribution of probands according to dental status

some respects it was representative of all workers bout 50 years f age.

Comparison with other series is, however of subordinate importance in the investigation f correlation of differ ent variables with sick absence Other aspects are of greater importance, three of which are dwelt n below

1. DISTRIBUTION OF THE MATERIAL WITHIN THE DIFFERENT VARIABLES

It is clear from Figs. 27–60 that the distribution of the diff rent variables was seldom normal. This makes the statistical evaluation difficult (see page 76). Some of the variables were classified and for these the distribution could n t, f course, be normal. (Types civil status, blood group)

The kewed distribution of some of the other variables is readily understood. Some examples f llow The distribution of certain variables, uch as sick absence, were J-shaped This also applies to absence f r reasons other than sick ness and some dietary habits. The riables referring t the w rking places w re not normally distributed either The demands f the work according t th Appendix and Figs 43 45 how kewed distribution. This also holds for the key occupations in Appendix. The distribution w not calculated fo the entire company but, ccording t data from the personnel ffice the distribution wa also probably skewed. This also pplies t th eval tion of the merits, though an ttempt wa made to secure a normal d stribution (See Appendix nd Fig. 46) In practice it was found that an inflation of the report had made tself felt

The reason why there were not so many who had been in the Company between 25 30 years w that so few had been mplo eed d ring the depression in the beginning f the 1930ies The di tribution of the ges of the parent would ha been better if the material had n t been di ided according t whether the parents were alive or not but according t the dates f birth of the parents

The distribution of body-build was only slightly but nevertheless distinctly skewed. This might be explained by the fact that the series consisted of a selected group of the population.

2. ARE THE DATA RELIABLE?

As mentioned in the introduction, as many data as possibl were obtained from the registers etc. Certain variables, however are based on the personal reports of the probands and others are the results of the clinical xamination. Some data in the registers may of course, also be erroneous, as was f und in the collection of the present series.

The official registers d not, however always give the data one wishes to have The G.S.I registers d n t, for example give sufficient inf rm tion on the stat of health of the wives (See p 81)

The data gi en by the proband on privat matters may be less reliable This pplies above all t the consumption of spirits. All reported that their consumption f spirits was low As far a teetotalism and moderat drinkers are concerned, the data may be regarded as reliable but not concerning those with problems due to alcoholism. However those with problems usually reported somewhat higher consumption than the others Among those with alcohol problems, howev there were some who reported that they were now teet tallers and that they belonged to some religious sect or t some tetotallers ssociation There is no reason to doubt their reports T check the reliability of the data, a comparison was made between the reported amount of pints consumed and the number of tots they drank pe week. (Fig. 41) If it be ssumed that a tot is 7.5 cl. (which is tandard measure t restaurants) the consumption of spirits reported was 4.6 liters per yea ccording t T ble 3... The

Matrix 3 *Heaviness of work correlated to pulse rate at 900 kpm/min.*

Heaviness Grade	N	N	Not examined
4	10	4	
3	51	35	
	45	61	15
1		2	
Mean value	2.6	2.4	..8
	≤ 150	> 150	Not examined

Pulse at 900 kpm/min

Matrix 4 *Muscle strength required correlated to shoulder thrust.*

Demands					Mean value
	2		14	14	58
5	3	11	13	5	51
50	13	35	36	*9	5
..5	10	16	10	8	48
Mean value	35	36	48	48	
		40	50	60	kg.

shoulder thrust

Matrix 5 *Demands on organs of locomotion correlated to evaluation of these organs.*

Demands grade						Mean value
3			3	1	38	4
	1	4	10	3	7	4 3
1			1	3	6	4..
Mean value	2.0	0	1 9	..3	..4	
	1		3	4	5	

evaluation grade

The differences found in the matrices were not tested for significance but it is clear that those who had a high pulse rate during the cycling test were doing work that was not quite so heavy those doing work requiring a large degree of muscular strength gave better result for the shoulder thrust and those doing work *requiring physical strength* gave better results on the average. This might suggest a certain tendency to adaptation to the requirements of the work.

DISCUSSION

Owing to the selection of the material (page 73) its distribution within the different variables was accounted for in detail. The main purpose of the study was to find correlations between the different variables and sick absence but the data given also permit comparison with other materials. However only few series of similar type have been published and comparison is difficult because the purposes of those studies and the variables analysed were not the same as in the present investigation. However SEGERSTEDT & LUNDQUIST reported roughly the same standard of living accomodation in the industrial centres they examined, and the standard of the houses of the workers was also lower than that of the salaried employees and most of the industrial workers had moved to the town from the country. This is in substantial agreement with what was found for ASEA. SEGERSTEDT & LUNDQUIST also studied the way in which the workers spent their free time as well as the conditions at the working places but they used different variables and a different technique for which reason comparison are hardly possible. Neither does the literature contain comparable data on consumption habits and body build. Suffice it here to say that in some respect the group studied resembled and in others differed from other groups and that in

some respects it was representative of all workers about 50 years of age.

Comparison with other series is, however, of subordinate importance in the investigation of correlation of differ ent variables w th sick absence. Other aspects are f greater importance, three of which are dw lt on below

1. DISTRIBUTION OF THE MATERIAL WITHIN THE DIFFERENT VARIABLES

It is clear from Figs. .7—60 that the distribution f the different variables was seldom n rmal. This makes the statistical evaluation difficult (see page 76). Some f the variables were classified and for these the distribution could not, of course, be normal. (Types: civil status, blood group)

The skewed distribution of some of the other variables is readily und rstood. Some examples f llow The distribution of certain variables, such a sick absence, were J-sh ped. This al-o pplies t absence for reasons other than sick ness and some dietary habits The variables referring t the working places were not normally distributed either The demand f the work ccording to the Appendix and Figs 43—45 how a kewed distribution. This also holds fo the key occup tions in Appendix. The distribution wa n t calculated for the entire company but, according t data from the personnel office the distribution w also probabl skewed. This also applies to the evalu tion f the merits though n ttempt was mad t secure a normal distribution (See Appendix and Fig 46) In practice it was found that an inflation f the report had m de tself felt.

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reported amount includes also the consumption during general holidays so that the values appeared to be in good agreement with those reported

The clinical examinations also have inherant sources of error In the present investigation the measurement of the *blood pressure during work* should be evaluated with caution because the method requires the probands to relax one of the arms during work The muscle factor is also influenced by the proband s technique This applies particularly to stretching of the back which was therefore rejected as a measurement of muscle factor In order to limit the sources of error as far as possible, each variable was only measured objectively by an ECG apparatus

3 SPREAD OF MATERIAL

The breadth of distribution was sometimes so small that it could not be decided whether the variable studied influenced sick ab ence or not. This holds for example for shift work, since only 11 of the probands were not working during ordinary hours It also applies to outdoor workers who were very few The consumption of cigarettes reported was low only 20 said that they smoked more than 10 cig /day and 7 more than 15 cig /day It is therefore less likely that cigarette smoking had influenced sick ab ence in this material

SUMMARY

Chapter XI gives an account of a *number of variables from the environments* during childhood and adolescence, family conditions habits during free time and dietary and drinking habits conditions at place of work, body build and hereditary factors and finally the physiological and clinical state of health Only few of the variables studied showed a normal distribution. Therefore not only the mean values and *the standard deviation but al o the* spread of the different variables are given *The* reason for the skewed distribution is discussed, as is the reliability of the data and it is shown that the distribation of some of the variables was too narrow to permit any conclusions

CHAPTER XII

ABSENCE

TECHNIQUE

Data on absence were obtained from the work cards f r the period 1 1 1956 — 31,12 1959 If a man is away from work, the f reman notes the reason fo absence under on of the 10 following headings.

1 *At work outside workshop*
2. *Holidays*
3 *Industrial injuri s*
4 *Other i juries*
5 *Disease other than according t 3 and 4*
6. *Pregnancy or military service (m l tary refresher course)*
7 *Temporary dismissal*
8. *Absence without permission*
9 *National service*
10. *I oluntary absenteeism*

Absence according to 3, 4 5 8, and 10 was anal sed and treated statistically

Overtime was also tudied. All absences w re noted with an ccuracy of 0 1 hour

The remaining categories of absence were not included f r the f llowing reasons. The material wa selected in such way th t bsence ccording to 1 w negligible If absence according t 3 4 and 5 happened to coincide with bsence according t 1 it wa noted under the headi gs f 3, 4 and

Absence cording t 6 or 9 did not occur d ring the period tudied.

A f r absence according to owing t lack of work during 193 and 1958, th foundries closed down for a short while during Christmas This affected 40 men of the present material Thus, during 19 7 they were without work f 54.25 hours, and in 1958 for 51.25 hours (total 105.5 hours) This implies 1.2 of the entire 4-year period This absence can therefore hardly have had any effect on absence because of 3 4 and 5

Absence under 3 4 and was recorded according to the norms given in Sick Absence Statistics (see Part I p 10) Use was m de of SAS 310 *i.e.*, the period prevalence rate for the entire observation period and SAS 311 *i.e.*, the average duration f completed or incompleted spells bserved, per person under observation. The time is given in hours As f absence according to subheading 5 SAS 311 was also cal culated for ea h year separat ly Absence ccording t 8 and 10, was recorded in analogy with SAS 311 was vertim

Absence supported by a medical certificat *i.e.*, absence recorded under 4 and 6 and reported to the C S I. and f more than 8 days d ration, wa grouped according to th disease responsible for the absence The diseases were grouped according to the classifica tion recommended by W H O The number of ick days per diagnostic group per 100 employees per year is given graphically Since this registration was founded on data from the G.S.I., the sick absence is given in days and inludes w kfree days.

8 arts

reported amount includes also the consumption during general holidays so that the values appeared to be in good agreement with those reported

The clinical examinations also have inherent sources of error In the present investigation the measurement of the blood pressure during work should be evaluated with caution because the method requires the probands to relax one of the arms during work. The muscle factor is also influenced by the proband's technique This applies particularly to stretching of the back which was therefore rejected as a measurement of muscle factor In order to limit the sources of error as far as possible each variable was only measured objectively by an ECG apparatus

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The breadth of distribution was sometimes so small that it could not be decided whether the variable studied influenced sick absence or not This holds for example for shift work, since only 11 of the probands were not working during ordinary hours It also applies to outdoor workers who were very few The consumption of cigarettes reported was low - only 20 said that they smoked more than 10 cig /day and 7 more than 15 cig /day It is therefore less likely that cigarette smoking had influenced sick absence in this material.

SUMMARY

Chapter XI gives an account of a number of variables from the environments during childhood and adolescence, family conditions habits during free time and dietary and drinking habits, conditions at place of work, body build and hereditary factors and finally the physiological and clinical state of health Only few of the variables studied showed a normal distribution Therefore not only the mean values and the standard deviation but also the spread of the different variables are given. The reason for the skewed distribution is discussed a is the reliability of the data and it is shown that the distribution of some of the variables was too narrow to permit any conclusions.

RESULTS

The average bsence in some categories is gi en in Table 39 The distribution of th absence is given in Figs. 61–65 Absence according t diagnoses available from certificates are given in Table 40 and Table 41 gives those persons who were aw y for more than 182 days

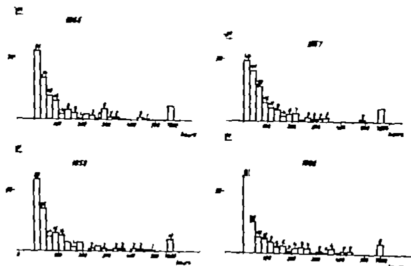


Fig. 61. *Distribution of probands according to sick absence in hours per year*

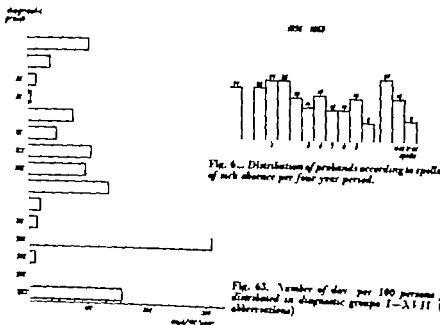


Fig. 6.. *Distribution of probands according to spells of sick absence per four year period.*

Fig. 63. *Number of day per 100 persons per year distributed in diagnostic groups I–XVII (See abbreviations)*

DEFINITIONS

Final day — The definition according to *SAS 03 (see Part I p 11)* was applied except for 03 e, where it is stated that the final day is the 182nd day This recommendation was not followed because the observation time was 4 years Those spells that lasted more than 182 days, are therefore accounted for in Table 4

3 *Industrial injury* — The definitions given in the law of 14.5 1954 § 6 were used

Industrial injury is to be understood as

a) injury owing to accident during work

b) injury otherwise caused by work and produced by the effect of substances or radiation energy and

c) injury not due to accident but caused by work and produced by the effect of one-sided unusual or unusually strenuous movements of locomotion, continuous repeated or unusual pressure of vibration of the machines or tools or of noise or infection. Injury is to be understood as physical injury — sun stroke heatstroke chills inflammation, as well as injury due to mechanical effect of at most a few days are always to be described as accidents To "accident during work are also ascribed accidents on the way to or from work, provided that the journey was undertaken because of or in association with work. The law of 29 10 1954 describes which injuries are to be understood as occupational injuries according to § 6 c)

4 *Other injuries* are of course injuries not classifiable as industrial injuries

5 Here all *absence because of disease* other than to injuries are recorded

It is not so easy to define the term disease The Social Welfare Committee of 1938 writes that "Disease is to be understood as any abnormal physical or mental state which has nothing to do with the normal process of life" JELLINEK (1960) says that a disease is what the medical profession recognizes as such

The person who decides whether he is disabled by a disease or not is as a rule the patient himself The Company follows the principles of the G.S I which implies that only absence of more than 8 days requires production of a medical certificate

8. *Absence with permission* — Leave of absence is to be understood as absence granted and not caused by any of the reasons given under 2—9 Leave of absence is granted by the foreman and should as a rule be applied for beforehand.

10 *Voluntary absenteeism* is to be understood as absence for no acceptable reason

Overtime is to be understood as the time exceeding normal working hours in a given week minus any absence during that week. Thus, if a person has been away one day and works overtime the following day overtime will only be registered as such if the number of hours he has worked exceed the normal number of working hours per week A normal working week in Sweden in 1956 and 1957 was 48 hours but in 1958 it was reduced to 47 hours and in 1959 to 46 Number of working hours during the 4 years covered by the present investigation is given below

Normal working time for the 4 years studied

1956 2248 hours
1957 2242 hours
1958 2222 hours
1959 2145 hours

RESULTS

The average absence in some categories is given in Table 39. The distribution of the absence is given in Figs. 61—65. Absence according to diagnoses available from certificates are given in Table 40 and Table 41 gives those persons who were away for more than 182 days.

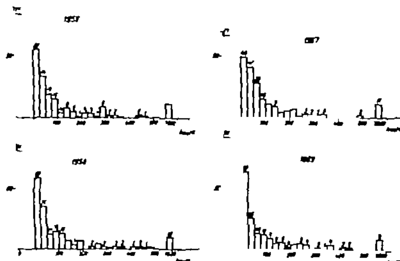


Fig. 61. *Distribution of probands according to sick absence in hours per year*

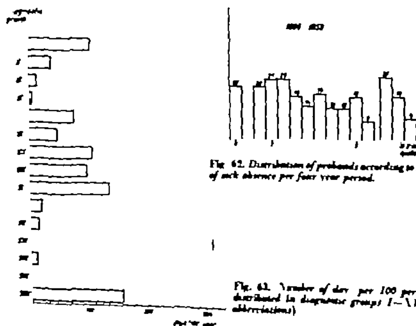


Fig. 62. *Distribution of probands according to spells of sick absence per four year period.*

Fig. 63. *Number of days per 100 persons per year distributed in diagnostic groups I—XVII. (See abbreviations)*

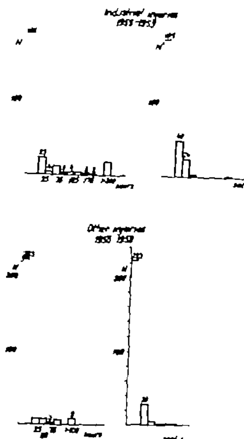


Fig 64 Distribution of probands accord ng to i juries.

Table 39 Arithmetical mean nd standard deviat on of bsence

Time in hours			M	S
3. Industrial injuries	SAS 311	19 6–59	5_.6	180.8
	SAS 310	1956–59	0 4	0.8
4. Other injuries	SAS 311	1956–59	15.1	63.
	SAS 310	1956– 9	0	0
5 Disease, excluding 3, 4	SAS 311	1956	82.5	161 9
		195	91.8	193.
		1958	89 3	13 4
		1959	87 6	190 1
		1956–59	316.5	528.8
	SAS 310	1956–59	6.8	6 0
8. Absence with permission	SAS 311	1956–59	90.3	93.6
10 Voluntary absenteeism	SAS 311	1956–59	_.8	11._
Overtime	SAS 311	1956–59	6	13.8

Table 40 Sick absence (3 4 5) classified according to medical certificate filled in in accordance with WHO nomenclature. Time in days. Absences of less than 8 days not included.

	Number of patients	days
I Morbi infectiosi et parasitarii		
002 Tuberculosis pulmonum	2	929
086 Rubeola	1	16
088 Herpes zoster	2	98
089 Parotitis epidemica	1	10
138.0 Lymfogranulomatosis benigna	1	10
II Neoplasmata		
223 Neoplasma benignum cerebri	1	399
III Morbi allergici, systematis endocrini, metabolismi et nutritionis		
252 Thyreot xicosis	1	95
253 Hypothyreoidismus	1	23
IV Morbi systematis haematopoetici et sanguinis		
293 Anaemiae non definitae	1	85
V Morbi mentis		
318 Casus psychoneurotici non definiti	20	784
VI Morbi systematis nervosi et organorum sensus		
361 Neuralgia trigemini	1	177
360 Paralysis facialis	1	23
391 Otitis media	1	9
395 Syndroma Ménière		262
396 Alii morbi aurис	1	34
VII Morbi organorum circulationis		
422.1 Arteriosclerosis myocardii	3	215
433.0 Interclusio fasciculi auriculoventricularis His	2	61
444 Hypertonia essentialis benigna	4	406
460 Varices venarum extremitatum inferiorum	10	334
461 Haemorrhoides	1	86
463 Thrombophlebitis extremitatum inferiorum	1	1
VIII Morbi organorum respirationis		
471 Sinusitis acuta	3	84
473 Tonsillitis acuta	3	31
475 Infectio itinerum respiratoriorum superiorum	14	230
481 Influenza cum aliis symptomatibus respirationis	16	243
493 Pneumonia non definita	6	210
500 Bronchitis acuta	6	193
519 Pleuritis	1	18
IX Morbi organorum digestionis		
540, 541 Ulcus ventriculi duodeni	17	1071
537 Sialolithiasis	1	13
543 Gastritis ac	1	14
560 Hernia abdominalis, obstructio non indicat	4	150
584 Cholelithiasis	5	165
X Morbi urogenitalium		
602 Calculus renis et ureteris	3	4
611 Prostatitis	2	79
613 Hydrocele testis	2	[illegible]
XII Morbi cutis et subcutis		
690 Furunculus	1	1
693 Dermatitis alia	4	138
XIII Morbi ossium et organorum locomotoriorum		
722 Arthritis, lumbago, alia et morbi similes	16	973
726.0 735 725.1 Lumbago, morbi cartilaginum intervertebralium, spondylosis deformans	35	2158
41 Synovitis bursitis, tendinitis aginitis	5	83
XIV Malformationes congenitae		
5? 1 Ren cysticus	1	107
XVII Injuries due to violence and poisoning	43	1628
(of which, head injuries with signs of involvement of meninges or brain)	(7	269)

Table 41 *Spells f mor than 18 days d ration.*

	`	D)
The pulm	1	199
	1	730
Neoplasma benign cerebri	1	399
Morbus Ménière	1	221
Hypertonia essentialis	1	322
Prolapsus nuclei pulposi	1	-60
Total		131
(Days more than 18*)		1039

ABSENCE DURING DIFFERENT YEARS

Chapter 8 gives the distribution of the number of spells of absence in this material, Fig 61 shows the distribution of the duration of absence during the individual years, and Table 39 gives the mean values found for absence Every where the level of sick absence was found to be remarkably constant This is in agreement with previous investigations such as that published by FORTUIN (1955) In Chapter 8 it was concluded that absence was not governed by chance, and it appears that each individual has a certain tendency to be away from work on a particular number of occasions with a particular total duration per year at least for the 4 years studied

Absence was greater during 1957 than during the other years when it was fairly constant (Table 39 Fig 61) It i clear from Fig that the increase was due mainly to absence of 25—200 hours with a peak at 50—100 hours Thi increase was undoubtedly ascribable to an epidemic of Asian influenza in Västerås during the last quarter of 195"

In the present 4-year material no tendency to increased ab ence with the years was noted

COMPARISON BETWEEN SICK ABSENCE AND SICKNESS

It is important not to confu e the term sickness with sick absence On medical examination, many disea es may be discovered which require treatment or observation and which cause pain of varying intensity but not sufficient to justify sick absence Thi may be illustrated by the following examples from the present material

Case No 71 Man aged 47 crane driver Since 2 years of age he had organic nervous disease interpreted a Morbus Little with progressive difficulties in walking These difficulties however did not prevent him from going to work (distance about 100 m) *though with difficulty and getting* up to the crane by lift During the observation period he had been on the sick list because of inguinal hernia duodenal ulcer gonarthrosis and for 3 short period becau e of upper respira tory tract infections None of these diagnosi had anything to do with hi organic nervous disea e

Case No 25 A caretaker aged 50 years and employed since 1955 As a sequal of neglected care during infancy he has severe rachitic changes with deformation of the skull, chest and spine During the observation period he had pneumonia on 4 occa ions and crural ulcer on one

Case 91 A truck driver aged 47 years employed since 1950 For 15 years he ha had open ulcers on *both lower legs* During the observation period he ha not been away from work because of these ulcers In the 1930ies he con ulted hi doctor about the ulcers but since treatment gave no *improvement* he did not consult any other doctors later *He*

also has psoriasis, fo which he has not been away from work. On the other hand he has been absent for all together 47 days because of myocardosis and for one month because of pneumonia.

Case N 199 A man, aged 51 employed since 1946 at th tool room tender Since 1937 he has had diabetes mellitus requiring insulin. He had n t been absent for a single hour during the observation period.

It should be added that chronic diseases ca se absence for limited periods in association with exacerbations or complicating tr ial diseases

DISTRIBUTIONS OF DISEASES AMONG DIAGNOSTIC GROUPS

On classification f data according to the different diagnostic groups, t will soon be realized that th diseases belonging t each group have little in common from an aetiological point of iew As pointed out by SJÖVALL, f example the headings of the chapters are not based n any uniform system. Sometimes they are based on etiological grounds, sometimes on anat mical. These headings have however been applied here since no bett r classification y tem is a ilable

In the discussion f circulatory disorders, it is often not realized or remembered th t this heading also includes aricose eins leg ulcers haemorrhoid and heumatic fever and not only diseases of the heart In the same w y diseases of the digesti tract includ also bdominal hernia, et

It is sometimes difficult t decid under which heading a diagnosis hould be placed. One and the same disease may be placed just as correctly under one heading as under another This is illustrated by the following examples

Low back pain may be given any of the following diagnoses.

723 1. Spondylarthrosis deformans s a pathological-anatomical diagnosis decided by roentgen examination.

726 0 Lumbago i a purely symp-t matic diagnosis.

73. Morbi cartilagineum intervertebralium is also a pathological-anatomical diagnosis.

363 Sciatica. In some cases, leg pain and neurological symptoms are predominant and then it is tempting to diagnose the case as neuralgia ischiadica.

Finally some doctors believe low back pain to be a manifest tion of psychic disease and then diagnose the case as ne of th diseases in Chapt r V The ame disease can thus be diagnosed differently by different doctors and the case placed under different num bers and even under different chapters

It is clear from Table 40 that no ttempt was made to distinguish between the different types of back trouble all of the cases were taken together All of the psychi disorders were also taken together under a single heading. As shown in Part I (Fig 19) the relat ve importance of the different diagnostic groups varies with the pa tients ages. Since the range of the patients ages in the various groups was wide it is possible that some diagnoses in some groups dominated in low age classes and others in higher age classes. It might therefore be worth while con idering which diseases were of greatest importance in classes ge studied (Fig 63)

I *Infectious diseases* — At this age infectious diseases are n t particularly common. Only 7 spell of absence in 4 years The pells were al-o short with the exception f . cases of pulmonary tuberculosis, which caused an absence f 979 days

II *Tumours.* — Neither did the tumours cause much absence in this age

There was only one case, a benign cerebral tumour and the long absence caused by the growth was due to post operative complications

III Only 2 cases of *endocrine disorders* were noted and no cases of allergy or nutritional disorders

IV *Blood diseases* were rare: only one case of secondary anaemia was noted

V *Mental diseases* were relatively common Since the absence was on the average, 40 days, this group was responsible for a fair amount of absence. The diagnoses in the medical certificate are, however vague It was therefore decided not to divide this group into sub-groups It might, however be mentioned that most of the cases were of mild nature

Case No 245 The patient was subjected to lobotomy in 1952 because of schizophrenia. He has no demonstrable mental symptoms On the other hand he has been absent because of gon arthrosis

VI *Diseases of the nervous system and organs of sense* are of less importance One protracted case of Morbus Ménière and one of trigeminus neuralgia however caused a relatively long period of absence

VII *Circulatory diseases* included no case of rheumatic fever or of *morbi rheumatici chronici cordis* (400—416) Myocardosis had caused absence in 3 cases and patients with benign hyper tension had also been away from work for fairly long periods In this material however the diseases of the veins were responsible for two thirds of the number of spells of absences and for 40 % of the total duration of absence

VIII *Diseases of the respiratory tract* — This group was dominated by diseases usually known under the name of infections of the upper respiratory tract *Asian influenza* occurred, as mentioned in the last quarter of 195 in Västerås It is remarkable that only 16 of the 262 in this group had influenza causing absence of more than 8 days Four fifths of the number of spell of absence and 59 % of the duration of absence in this group were due to infections of the upper respiratory tract It might also be mentioned that of the 6 absences because of pneumonia, case No 4 was responsible for 4 of them

IX *Diseases of the digestive tract* — Peptic ulcer was a *prominent disease* and was responsible for two thirds of the spells and *for 75 % of the duration of* absence The rest was due to an equal extent to *abdominal hernia and gall* stone

X *Diseases of the urogenital tract* were of less importance

XII *Diseases of the skin* caused hardly any absence Of those who sought advice at the health office diseases of the skin played a much greater role than what might be supposed from this material. Much work in the Company is liable to injure of the skin, *e g.*, machine work with cooling fluid handling of different sorts of plastics material, gla fibre, etc It is probable that the present material included only a relatively *small* number of people doing such work.

XIII *Diseases of the skeleton and organs of locomotion* con tituted a group responsible for most absence in thi material This applies to both the num ber and the duration of absence Diseases of the back represented two third of the cases and 70 % of the duration in this group Back pain alone wa respon ible for 20 % of the total duration of absence

XIV *Congenital malformations* — One man had cystic kidneys—fairly advanced with threatened uraemia He ha been given light work and except for short absences because of hi disease he ha been able to work during the whole observation period

XVII. *Injury due to violence and poison ng (Fig 61)* — One might expect this diagnostic group to play prominent role in group of w rkers. Nevertheless only 43 of the accidents caused absence f more than 8 days d ring the 4 observation years.

Summarizing back trouble was of greatest importance and was respons ble f r 20 % of the duration f bsence after which came ccidents and peptic lcers with roughly 10 ea h, and *infections of the upper respirat ry tract* with about 5 %. If also short pells f absence were included, the absence d t infections of the upper respirat ry tract would have been much higher (see part II p 59)

COMPARISON WITH TOTAL ABSENCE IN COMPANY

The level f SAS 310 (the period prevalence rat) nd SAS 311 (the verage duration of spells) in each f the groups were the sam a f r the entire Company SAS 310 was not tudied f t her w rkers, but the salaried male employees i corresponding age classes had SAS 310 1.6 per year and in thi material it wa 1 per year The difference between bsence f salaried employees and workers respectively was th due t th severity rat nd not t the period prevalence rat at lea t not in this ge class.

The bservation period f 4 years gives a more detailed pict re f the diagnosis than what wa f und f the entire Company which was t died for only one ear Otherwise the tw distribution among diagnostic groups d n t differ ubstantially from one anothe

Comparison howed th t despite the trict criteria s tisfied by thi material, the findings did not differ ubstantially from what wa found for the Company whole

ABSENCE WITH PERMISSION

The *duration of absence with permission* was equal t 22 of sick absence The number of absences was not calculated, but, judging from rand m samples, it wa probably about 10 times higher than the period prevalence rat because of disease Th distribution of absence with permi sion is given in Fig 65

VOLUNTARY ABSENTEEISM

Volunt ry absenteeism was relat vely little (Fig 65) Only 7 persons or 2.5 % of the material, had been away from work for more than 70 hours without any acceptable reason.

OVERTIME

The a erage o ertime during the 4-year period was .7 hours. The distribution is given in Fig 63 Absence without permi ion, voluntary bsenteeism and vertime are of interest if correlated with sick bsence.

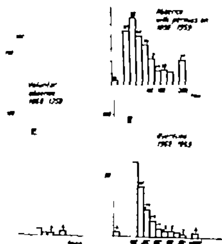


Fig 6.. *Distribution of probands according to other absence and overtime*

There was only one case a benign cerebral tumour and the long absence caused by the growth was due to post operative complications

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XVII. *Injury due to violence and poisoning* (Fig 64) — One might expect this diagnostic group to play prominent role in a group of workers. Nevertheless, only 45 of the accidents caused absence of more than 8 days during the 4 observation years.

Summarizing, back trouble was f greatest importance and was responsible for 20 % of the duration of absence, after which came ccidents and peptic ulcers with roughly 10 % each, and infections of the upper respiratory tract with about 5 %. If also short spells f absence were included, the absence due to infections of the upper respiratory tract would ha e been much higher (see part II p 59)

COMPARISON WITH TOTAL ABSENCE IN COMPANY

The level f SAS 310 (the period prevalence rate) and SAS 311 (the average duration of spells) in each of the groups were the same as f the entire Company SAS 310 was not studied f t her workers, but the salaried male employees in corresponding age classes had SAS 310 1 6 per year and in this material it was 1 7 per year The difference between absence of salaried employees and workers respectively was thus due t the severity rate and not to the period prevalence rate, t least not in this age class.

The observation period of 4 years gives a more detailed picture f the diagnosis than what was found for the entire Company which was tudied f only one year Otherwise, th two distributions among diagnostic groups do not differ s bstantially from one another

Comparison howed that despite the strict criteria satisfied by this material, the findings did n t differ substantially from what was found for the Company a whole

ABSENCE WITH PERMISSION

The duration of absence with pe mission was equal to 22 of sick absence. The number of absences was not calculated, but, judging from random samples, it was probably about 10 times high than the period prevalence rate because of disease The distribution f absence with permission is given in Fig 65

VOLUNTARY ABSENTEEISM

Voluntary absenteeism was relatively little (Fig 65) Only persons, or 2.5 % f the material, had been way from work f r more than 20 hours without any acceptable reason.

OVERTIME

The average vertime during the 4-year period was 2 hours. The distribution is given in Fig 65 Absence without permission, voluntary absenteeism and overtime are of interest if correlated with sick absence

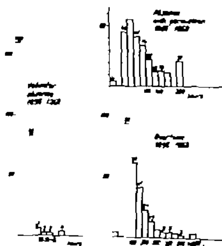


Fig 65. *Distribution of probands according to other absence and overtime.*

SUMMARY

Sick absence was fairly equal in 1956 1958 and 1959 It was somewhat higher in 1957 because of an epidemic of influenza

The difference between sick absence and sickness was exemplified

The main diagnoses responsible for the absence were found to be back pain injuries diseases of digestive tract and respiratory tract infections

The absence in the group studied was the same as in the entire Company

Absence with and without permission was of little interest

CHAPTER XIII

CORRELATIONS BETWEEN SICK ABSENCE AND OTHER VARIABLES DESCRIBED IN CHAPTER XI

W th the method given on page 75 correlation was found between sick absence and ther variables Table 4. gives all the variables with $p < 0.05$ The table also shows whethe the correlation was positive or negative.

I SICK ABSENCE EXCLUDING INJURIES

SIGNIFICANT CORRELATIONS WITH χ^2 METHOD

The closest correlation with sick absence excluding accidents was found for the ariables "merit presence" "offences during drunkenness" "consumption of drugs" "working capacity t time of examination" "evaluation of organ of locom tion" and "choice of occupation decided by health" (Fig. 66) The correlation appears t be natural except regarding the number of offences during drunkenness Th merit presence is influenced by the numbe f waiting days (three) in the vent f sick absence It is also fairly bvious that the con sumption of drugs is greater among indi duals w th s ck absence that those whose choice of w rk was decided by their poor tat f health were away from work more and that ck absence should be higher among those in whom clinical examination revealed low physical perf rmance

DIVISION OF MATERIAL INTO TWO GROUPS ACCORDING TO CONSUMPTION OF ALCOHOL

The correlation between drunkenness and sick bsence does not appea to be so n tural. It is clear from Fig 66 that not only those with offences during drunkenness had high sick absence but also those with alcohol problems but no known offence These four were registered separately If we take those 5. persons who were graded according to *drinking habits* (p 91) as grade c, d and e, it will be found that SAS 310 was 11 0 and that SAS 311 was 41 h urs, .e SAS 310 was twice and SAS 311 3.1 times greater than f grades and b Since the group is large (20 % of entire series) and th difference in absence considerable, on might imagine that it might influence the correlation between absence and the ther variables.

Theref re those individuals belonging to grade c, d or w re afterwards dealt with separately in th group called B The other 200 re called group A. In this way it was possible to ascertain whether any correlation was present with and without group B and secondly whether there is an increased risk f belonging t group B fo any of the variables i.e an increased risk for drunkenness o other alcoholic problems.

One might of course also imagine that factors ther than those in group B might influence the different variables. In the present investigation, however the author contented himself with group B since, according t Table 42, it is of

SUMMARY

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The difference between sick absence and sickness was exemplified

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The absence in the group studied was the same as in the entire Company

Absence with and without permission was of little interest

	1	2	3	4	5	6
Demands on organs of locomotion			6.1 + $p < 0.02$			
Conversation possible			6.1 + $p < 0.02$			
Noise. Average level			7.1 + $p < 0.01$			
Max. level			5.8 + $p < 0.02$	5.2 + $p < 0.05$		
Employed in Company					4.2 + $p < 0.05$	
Choice of job influenced by health?		16.7 + $p < 0.001$				
Size of town brought up in			7.8 − $p < 0.01$			
Athletics					5.7 +	
Open air life	8.9 − $p < 0.01$					
Alcohol consumption					7.8 + $p < 0.01$	4.4 + $p < 0.05$
No. offences of drunkenness	5.9 + $p < 0.02$	15.8 + $p < 0.001$	5.6 + $p < 0.02$			
Smoking factor			7.0 + $p < 0.01$			
Drug consumption	12.5 + $p < 0.001$	20.9 + $p < 0.001$				
Muscle factor: shoulder thrust			4.3 − $p < 0.05$			
Skinfold thickness subscapular			4.3 − $p < 0.05$			
Parathoracic			4.3 − $p < 0.05$			
F factor according to Lindegård		5.7 − $p < 0.02$				
Heart vol.	3.9 − $p < 0.05$					
Pulse on exertion (900 kpm/m)	4.0 + $p < 0.05$					
Working capacity time of exam	5.9 − $p < 0.02$	21.7 − $p < 0.001$				
State of organs of locomotion		22.6 − $p < 0.001$				
Injuries before 1956		5.3 + $p < 0.05$	40.2 + $p < 0.001$	55.4 + $p < 0.0001$	38.2 + $p < 0.001$	

occupation decided by [illegible] health" ($p < 0.001$). The correlation between sick absence and "presence" "working capacity" etc. was discussed on page 124.

The negative correlation between SAS 311 and quantity appears to be probable and natural since long absence must decrease production.

The two remaining correlations are thus SAS 310—theory and SAS 310—judgment and initiative. These two variables are strongly correlated with one another as pointed out by BROWNER (1951). In the present material the $\chi^2 =$ 113 (sic) as the two variables have practically the same number of points for each individual in the material. "Theory" was correlated with a number of other variables (Table 43).

Table 4... Variables correlated with sick absence according to χ^2

	Sick absence 1956—1959 excl. injuries SAS 310	Sick absence 1956—1959 ex l. injuries SAS 311	Industrial injuries 1956—1959 SAS 310	Indu trial injuries 1956—1959 SAS 311	Othe injuries leave (1956 19 9) SAS 310	Othe inj ri lea (19 6—1959) SAS 311
	1		3	4	5	6
Requi ements f job						
Theory	9 6 + p< 0 01					
Practice	6 9 + p< 0 01	4 0 + p< 0.0				
Judgement and initiative	9.3 + p< 0 01	4.3 + p< 0 05				
Skill and dexterity	4 8 + p< 0 05					
Responsibility f r thers safety			1 1 + p< 0 05			
Physical stamina	5.5 — p< 0 0		8.9 + p< 0 01			
Psychic tamina	4 7 — p< 0.0					
Environments			1 7 + p< 0 01			
Merit						
Quantity	5 8 — p< 0.0*					
Quality			5 5 — p< 0 0*	1 6 p 0 05	1.6 p 0.05	
Presence	9 3 — p< 0 01	10.6 — p< 0 01				
Heaviness of work	4.6 — p< 0.05		1 + p< 0 01			

the greatest importance apart of course from self-evident correlations

As mentioned on page 76 the χ^2 test is unreliable if the distribution of the variables is not normal. Therefore with the method described on page 76 those variables were studied which were found to be correlated according to columns 1 and 2 in Table 42 The material wa divided into groups A and B SAS 310 and SAS 311 respectively were calcu lated for each grade of the variable separately and the difference between the grades with lower and higher values respectively of SAS were tested f r significance with the t test The result are exemplified in Fig 66—67

CONDITIONS AT PLACE OF WORK

Of the variables included in the tal le there are three which in group A are correlated with SAS 310 namely "m rit presence" ($p < 0.005$) "demand of work: theory" ($p < 0.005$) and "ju lg ment and initiative" ($p < 0.001$) and with SAS 311 the following three variables "merit presence" ($p < 0.01$) and "quantity" ($p < 0.0$) and present

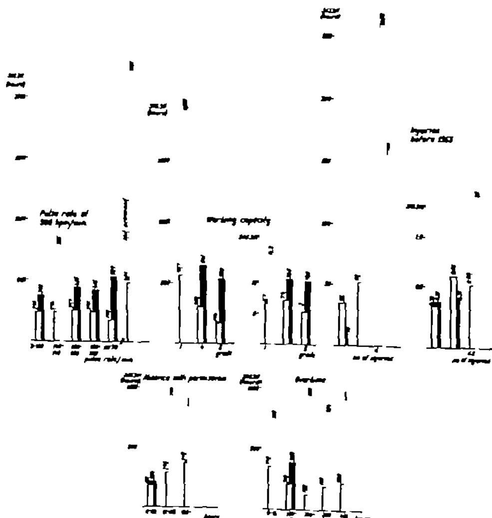


Fig. 67

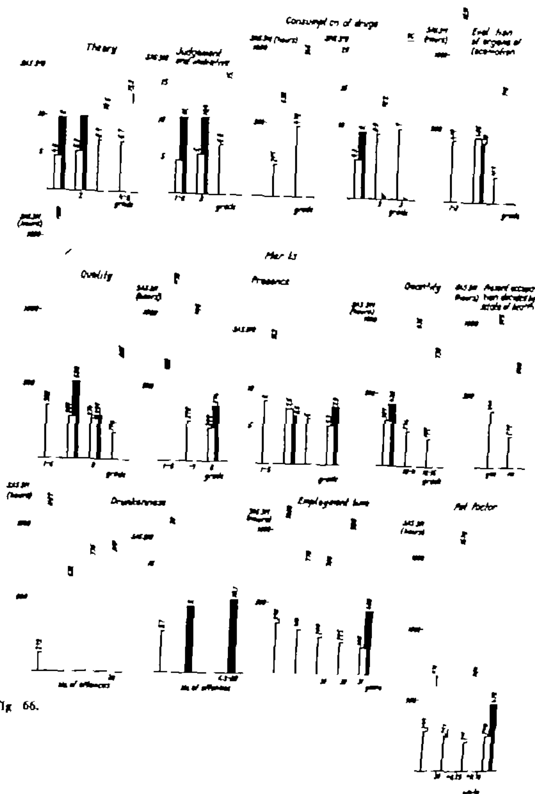


Fig 66—6 *Correlation between sick absence and other variables described in chapter XI. SAS 310 period prevalence rate. SAS 311 Average duration of completed or incompleted spells observed per person under observation. Blank columns group A. Filled columns group B.*

tively correlated with SAS 310, if group B be included. As to heavy work, only the healthiest could be employed so that those with a high theory factor should have a relatively lower factor for tate of health.

These two explanations may seem a little farfetched and it might be wise to regard the correlations with caution until they ha e been verified in a larger material. Moreover the correlation is of less importance f r the health of the workers and fo the economy of the Company because it is not a question of duration f absence but f th number of absences. The difference between extreme grades 1 and 4 is only 2.1 spells in 4 years

ACTIVITY DURING FREE TIME (Table 42) shows a correlation only with SAS 310

The only correlation found fo free time was that between open air life and SAS 310 It ppears but natural that persons who like to be out in the pen air during their free time are healthier or that those who re often ill cannot stand being out of doors so much In group A the difference was n t significant

CONSUMPTION HABITS

The correlation between SAS 310 and SAS 311 with the number of convictions for drunkenness and consumption of drugs were discussed on page 125

STATE OF HEALTH FROM 15 YEARS OF AGE UNTIL 1954

A negativ correlation was found between inj res and SAS 311 (Table 42) The correlation was, however not significant after limination f group B

BODY BUILD

A negati e correlation was found between the fat fact ccording to LINDEGARD and SAS 311 (Table 42) The correlation was n t significant in group A (Fig 66) This also holds for the heart lume Body build variables are discussed in further detail in Chapter XIV

PHYSIOLOGICAL AND CLINICAL DATA

The pulse as measured in 900 kpm./min. was positively correlated with SAS 310 i.e persons in a relatively poor physical condition had a greater risk of being absent more often than the remainder The correlation was, how ever not significant for group A W rking capacity at the time of the examination was found to be correlated with SAS 311 in group A but not with SAS 310 (See Fig 67)

OTHER SIGNIFICANT CORRELATIONS

As mentioned on pages 76 and 110 the χ^2 test is not sufficient for analysis of this material and the influence of group B should be eliminated. Groups A and B were therefore also distributed among all variables included in Chapter 0 and correlated with SAS 311 The significance f the correlations was tested by the t test In addition to the correlations already mentioned the following relations were found.

CONDITIONS AT PLACE OF WORK

M rits. — As is apparent from Fig. 66 a significant difference was found between the lowest and highest number of points for quality ($p < 0.05$) Th reason why no correlation was found with the Facit EDB was probably that the distribution of the variables was kewed. This variable is correlated with "merit : quantity" ($\chi^2 = 37.7$), which makes the correlation easier to under tand

The duration of employment in the Company showed a clear difference between the oldest and the youngest (Fig. 66) ($p < 0.05$). It was interesting to note that those who had been in the employ of the Company for more than 30 years were healthier than those who had been employed for less than 10 years

Table 43. *Variables correlated with requirements of work theory*

	χ^2 Df = 1	P (less than)
Requirements of job judgement and initiative	+113.1	0.000001
" " " skill and dexterity	+ 86.7	0.000001
" " " economic responsibility	+ 17.9	0.001
" " " leadership	+ 23.2	0.001
" " " physical exertion	− 4.5	0.05
" " " mental strain	− 39.9	0.001
Heaviness of work	− 4.0	0.05
Merit quality	+ 4.[illegible]	0.05
" knowledge	+ 13.8	0.001
Wages	+ 19.8	0.001
School education	+ 11.1	0.001
Athletics	+ 7	0.01
Indoor hobbies	+ 5.6	0.0[illegible]
Smoking pipe	+ 3.9	0.05
Size of flat in m²	+ 6.3	0.0[illegible]
Tibial length	− 4.1	0.05
Length factor	− 6.7	0.01
Shoulder thrust	+ 4.2	0.05
Impairment of hearing	− 4.1	0.05
Working capacity at time of exam.	+ 6.5	0.0

As shown in Chapter II it is widely believed that higher education is associated with lower sick absence. This is not directly compatible with the *finding here that the number of absences* was higher when the demands on theoretical knowledge in the workshop were high. The difference between grades 1 and 4 was that grade 1 requires elementary school knowledge and grade 4 also knowledge corresponding to three years at an industrial school. None of the members in the group studied had been to any such school. *The knowledge* they required had instead been obtained through practice and through private study. To understand the correlation between theory and *SAS 310 Table 43* is of less value because only "working capacity at time of examination" was found to be correlated with SAS 310. *Neither is the correlation easy to explain* if we study which key professions are represented in grade 1 and grade 4 for the theory factor (see *Appendix*).

The following explanations may be considered.

1. The demands of the work might be too high for the workers. According to CRUICKSHANK (cit. MINDUS 1956) *there is an optimum for mental tension.* In this case one might imagine the demand in grade 4 to be too high, since they require higher knowledge than the worker really has. *On the other hand,* the material might also include some individuals who are really intelligent but *had not had the opportunity to study and therefore been obliged* to take an occupation in the workshop where they have been given a job with relatively high theoretical demands but which are *nevertheless too low to be stimulating.*

2. Those who have work with relatively high theoretical demands might represent a selection with less good health. "Heaviness of work" and "physical exertion" are lightly negatively correlated with theory (See *Table 43*). These two variables *are in turn nega*

correlation between sick absence and absence with permission, which might be interpreted as a sign that sick absence constitutes a part of a general tendency to be away from work. This correlation, however permits no definite conclusions because absence with permission was granted for several reasons: public duties, personal problems which had to be solved during working hours etc. (No attempt was made to find out reasons why the workers had absence with permission.)

Of the merit variables "presence" and "quantity" may be regarded as self evident. This, however does not apply to "quality". However the merits are closely correlated with one another and this is probably enough to explain also the correlation with quality.

None of the correlations found in group A can be regarded as being of aetiological significance for sick absence. Group B is discussed in further detail in Chapt. XV.

II INDUSTRIAL INJURIES AND OTHER INJURIES

This study was designed to investigate sick absence, and the choice of variables was made accordingly. However absence because of industrial injuries and other accidents was also noted, so that it is possible also to give an account on those correlations of and with χ^2 on the Facit EDB (Columns 3—6 in Table 42). The correlations are also given graphically in Figs. 68—71 where the material is divided, as usual, into group A and group B. The correlation of group A was not tested by the t test for significance, nor were occupational diseases studied for any correlation with other variables than those included in Table 42.

During the 4 years covered by the investigation only 77 workers had had industrial injuries and 34 had other injuries, which caused an absence of more than 1 hour i.e. 29 % and 13 % of the material, respectively. Thus, especially for other injuries the material was very small.

Figs. 68—71 give the numbers of workers, in per cent of the material, with industrial injuries and other injuries during a 4-year period as well as absence expressed as SAS 310 and SAS 311.

WORKING CONDITIONS

"Heaviness of the work" "physical exertion" "environments" "speech interference" and "noise" are correlated with one another (Table 44).

All of these variables show the same tendency namely that the risks of occupational injury (disease) increases with relatively high degree of the variable. It is perhaps but natural that workshop environments with heavy, dirty noisy work increase the risk of occupational disease. All these variables were found to be correlated with SAS 310 i.e. number of occupational injuries but only maximal noise level was found to

Table 44. Correlations with χ between some variables at working place. Df = 1

	1	2	3	4	5	6	7
1. Heaviness of work		+ 73.1	+ 1 .0		+ 9.6	+ 11.3	+ 67.6
2. Physical stamina			+ 43.4		+ 16.7	+ 21.0	+ 49.2
3. Environments				+ 32.9	+ 10.2	+ 21.2	+ 15.2
4. Possibility to converse					+ 11.5	+ 5.8	
5. Average noise						+ 55.4	+ 15.0
6. Max. noise							+ 13.1
7. Skill required by work							

PHYSIOLOGICAL DATA

Pulse at 900 kpm/min (Fig 67) Those who could not manage this test were away from work more than the remainder Table 37 gives the reasons why these workers could not manage the test. It also leaves the impression that this group should have a higher sick absence It is remarkable that no difference was found in sick absence between individuals with a high and low pulse rate on physical exertion

OTHER ABSENCE

— SAS 311 was also correlated with other types of absence

Absence with permission — It was found that a long total absence with permission was correlated with a high sick absence ($p < 0.01$) (Fig 67)

Overtime — Judging from Fig 67 sick absence was lowest among those who had a moderate amount of overtime The difference between short and moderate overtime was significant ($p < 0.001$) but not between moderately long and long overtime It was interesting to observe that the 21 in group B who had more than 200 hours overtime had such a high sick absence compared with the other half of group B This correlation between overtime and sick absence does not warrant any definite conclusion because the opportunities the man have of putting in extra hours varies from time to time and from workshop to workshop Nevertheless it appears that the group with a relatively long total period of sick absence cannot manage so much overtime *On the other hand*, one might imagine that those who have been ill wish to do overtime to cover the losses sustained by the illness This would then apply above all, to those in *group B who had a long total duration* of sick absence Judging from the present material, overtime does not increase the risk of sick absence

COMMENT AND SUMMARY

After it had been shown that the variable in "convictions for drunkenness" is strongly correlated with sick absence excluding accidents the material was divided into two groups From the variable evaluation of drinking habits" grades a and b formed group A and the remainder i.e c, d and e group B Group B thus includes all who have on some occasion been convicted for drunkenness and the others who are known to be abusers of alcohol Group A was then studied for any correlation with sick absence *The significant correlations* thereby found are as follows

1 Variables which by their nature should reasonably be correlated with SAS 311

Merits presence

Choice of work dictated by state of health

Physical performance at time of examination

Evaluation of organs of locomotion

Consumption of drugs

Pulse at 900 kpm/min., that part of the material which could not perform the stipulated work compared with that which could

2 Correlation with other absence

Correlation between SAS 310 and SAS 312

Absence with permission

Overtime

3 Other correlations

Merits quantity and quality

Presence

Duration of employment in Company

SAS 310 also gave a correlation with the requirements the work placed on the workers (theoretical knowledge sense of judgment and initiative)

The correlation given under sub-heading 1 require no comments

It was interesting to find the distinct

correlation between sick absence and absence with permission, which might be interpreted as a sign that sick absence constitutes a part of a general tendency to be away from work. This correlation, however, permits no definite conclusions because absence with permission was granted for several reasons: public duties, personal problems which had to be solved during working hours, etc. (No attempt was made to find out reasons why the workers had absence with permission.)

Of the merit variables "presence" and "quantity" may be regarded as self evident. This, however, does not apply to "quality". However the merits are closely correlated with one another and this is probably enough to explain also the correlation with quality.

None of the correlations found in group A can be regarded as being of aetiological significance for sick absence. Group B is discussed in further detail in Chapter XV.

II INDUSTRIAL INJURIES AND OTHER INJURIES

This study was designed to investigate sick absence and the choice of variables was made accordingly. However, absence because of industrial injuries and other accidents was also noted, so that it is possible also to give an account on those correlations found with χ^2 on the Facit EDB (Columns 3—6 in Table 4). The correlations are also given graphically in Figs. 68—71 where the material is divided, as usual, into group A and group B. The correlation for group A was not tested by the t test for significance nor were occupational diseases studied for any correlation with other variables than those included in Table 42.

During the 4 years covered by the investigation only 77 workers had had industrial injuries and 34 had other injuries, which caused an absence of more than 1 hour, i.e. 29 % and 13 % of the material, respectively. Thus, especially for other injuries the material was very small.

Figs. 68—71 give the numbers of workers, in per cent of the material, with industrial injuries and other injuries during a 4-year period as well as absence expressed as SAS 310 and SAS 311.

WORKING CONDITIONS

"Heaviness of the work", "physical exertion", "environments", "speech interference" and "noise" are correlated with one another (Table 44).

All of these variables show the same tendency, namely that the risks of occupational injury (disease) increases with relatively high degree of the variable. It is perhaps but natural that workshop environments with heavy, dirty, noisy work increase the risk of occupational disease. All these variables were found to be correlated with SAS 310 i.e. number of occupational injuries but only maximal noise level was found to

Table 44. Correlations with χ^2 between some variables at working place. Df = 1

	1	2	3	4	5	6	7
1 Heaviness of work		+ 75.1	+ 17.0		+ 9.6	+ 11.5	+ 67.6
2. Physical exertion			+ 45.4		+ 16.7	+ 21.0	+ 49.2
3. Environments				+ 32.9	+ 18.2	+ 21.2	+ 15.2
4 Possibility to converse					+ 11.5	+ 3.8	
5. Average noise						+ 55.4	+ 15.0
6. Max. noise							+ 13.1
7 Skill required by work							

PHYSIOLOGICAL DATA

Pulse at 900 kpm/min (Fig 67) Those who could not manage this test were away from work more than the remainder Table 37 gives the reasons why these workers could not manage the test It also leaves the impression that this group should have a higher sick absence It is remarkable that no difference was found in sick absence between individuals with a high and low pulse rate on physical exertion.

OTHER ABSENCE

— SAS 311 was also correlated with other types of absence

Absence with permission — It was found that a long total absence with permission was correlated with a high sick absence ($p < 0.01$) (Fig 67)

Overtime — Judging from Fig 67 sick absence was lowest among those who had a moderate amount of overtime The difference between short and moderate overtime was significant ($p < 0.001$) but not between moderately long and long overtime It was interesting to observe that the 24 in group B who had more than 200 hours overtime had such a high sick absence compared with the other half of group B This correlation between overtime and sick absence does not warrant any definite conclusion because the opportunities the man have of putting in extra hours varies from time to time and from workshop to workshop Nevertheless it appears that the group with a relatively long total period of sick absence cannot manage so much overtime On the other hand, one might imagine that those who have been ill wish to do overtime to cover the losses sustained by the illness This would then apply above all to those in group B who had a long total duration of sick absence Judging from the present material overtime does not increase the risk of sick absence

COMMENT AND SUMMARY

After it had been shown that the variable in convictions for drunkenness" is strongly correlated with sick absence excluding accidents the material was divided into two groups From the variable evaluation of drinking habits" grades a and b formed group A and the remainder i.e c, d and e, group B Group B thus includes all who have on some occasion been convicted for drunkenness and the others who are known to be abusers of alcohol. Group A was then studied for any correlation with sick absence The significant correlations thereby found are as follows

1 Variables which by their nature should reasonably be correlated with SAS 311

Merits presence

Choice of work dictated by state of health

Physical performance at time of examination

Evaluation of organs of locomotion

Consumption of drugs

Pulse at 900 kpm/min., that part of the material which could not perform the stipulated work compared with that which could

2 Correlation with other absence

Correlation between SAS 310 and SAS 312

Absence with permission

Overtime

3 Other correlations

Merits quantity and quality

Presence

Duration of employment in Company

SAS 310 also gave a correlation with the requirements the work placed on the workers (theoretical knowledge sense of judgment and initiative)

The correlation given under sub-heading 1 require no comment

It was interesting to find the distinct

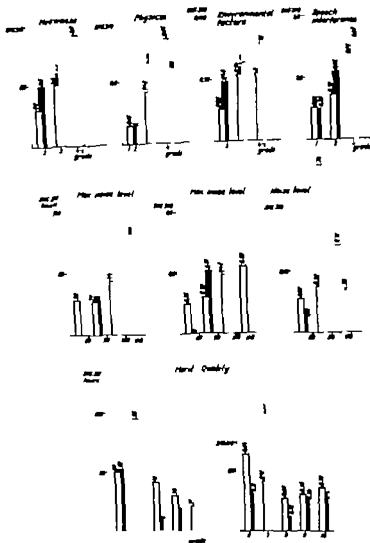


Fig. 69 Correlation between industrial injuries and other variables described in chapter XI SAS 310 period prevalence rate SAS 311 Average duration of completed or incompleted spells observed per person under observation. Blank columns group A Filled columns group B.

group A and in group B One might imagine that certain people are by n ture more liable t meet with accidents than others But then again in the present material it cannot be denied that those who had ccidents were doing risky w rk.

be correlated also with the duration of absence (Figs 68—69)

The evaluation of the merits was found to be correlated only with the quality of the performance of the workers which however was found to be correlated both with the number of industrial injuries and loss of time and number of other injuries Figs 68—69 show that this tendency exists also after elimination of group B

The duration of employment showed a correlation with the number of other injuries the risk of accidents decreasing with increasing duration of employment The difference was however so small that it was not significant for group A by itself

This also applies to the average wages which according to Table 18 showed a negative correlation with the number of occupational injuries

ENVIRONMENTS DURING CHILDHOOD

A negative correlation was found between the size of the town in which the workers had been brought up and the number of industrial injuries i.e. those who came from small places had a greater number of accidents (Table 4*) On closer analysis it was found that the risk was greatest for those who came from medium-sized towns somewhat less for those who came from villages and least for those who came from large towns i.e. in this material from Västerås The material particularly in the intermediate group was too small to allow of any definite conclusions in this respect

The number of other injuries was as might be expected higher among those who were active members of a sport club The material was small (Figs 70—71)

STATE OF HEALTH FROM 15 YEARS OF AGE UNTIL 1956

A definite correlation was found between the number of accidents before 1956 and the number 1956—1959 (Figs 68—69) The tendency was clear in

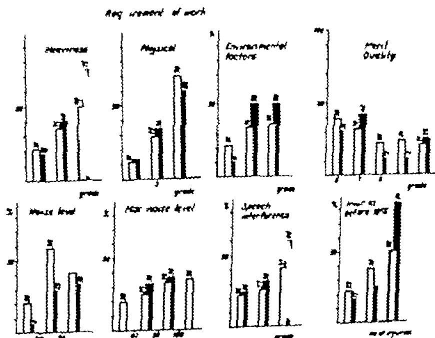


Fig 68 Correlation between number of probands with industrial injuries ( ) 1956—1959 and different variables described in Chapter XI Blank column group A Filled col mns group B

and parathoracic skin fold (Table 42). In all of these cases the correlation was negative. On closer inspection it was found that the correlations were not linear and that the differences in group A were fairly small. Since a correlation was found only for the muscle f ctor and fat factor no conclusions are warranted until the correlations have been con firmed in a larger material.

PHYSIOLOGICAL AND CLINICAL DATA

Here a correlation was found only between the number of other injuries and pulse rate t 900 kpm /min. The correla tion was d e to group B in which those who were in relatively good condition had most accidents. Among the .5 wh could not perform the test, nly one had had an accident, and h belonged to group B.

DISCUSSION

The ca se of accidents is at present receiving much space in the literature and accidents are ften ascribed t "th human factor". A point that has recei ed much attention in recent years is the occurrence f persons who are more liable to ha e accidents than thers (e.g. HAGBERG 1960, FORSSMAN 1961). The present in estigation incl ded too small a number f ccidents to permit any contribution t this debat. Summarizing however greater correlation was found between ccidents and conditions t wo king place than with body build, hab t and home en ironments.

A signif cant correlation was, however fou d between ccidents before and after 1956. This may be due t the individual ha ing more risky work, but may also be interpreted as a tendency to ccidents. If the material really included some people who were more inclined to have accidents than thers, the risk of meeting with an accident aft r working hours would be greater for those who had industrial injuries than f r thers. As mentioned, the material included 71 individuals with industrial inj ries (ii) and 30 with ther injuries (oi). 14 had both industrial and other injuries. If the risk of industrial injuries and ther injuries were independent, we should find P_{ii+oi} to be

$$\frac{71 \times 30}{260 \times 260} = 3.35\ \%$$

The value found was

$$\frac{14}{260} = 5.4\ \%$$

The difference is n t significant, so that this m terial does not support the theory of some people being more inclined t meet with accident than others. This also holds for each group separately.

SUMMARY

Different variables were studied f r any correlation with industrial injuries and other injuries. In the present material the risk of accident was greater at w king places with heavy, dirty and noisy work. The material does not support the theory f some people being especially inclined t meet with accidents. The material is not large enough t warrant any conclusions concerning tendencies to accidents, and the number of ccidents were studied only to lucidat any connection with sick absence.



Fig 70 Correlation between other injuries and different variables described in Chapter XIII SAS 310 period prevalence rate SAS 311: Average duration of completed or incompleted spells observed per person under observation.

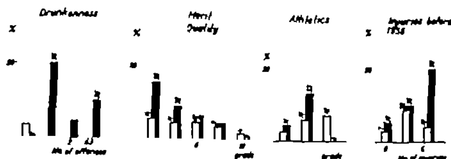


Fig 71 Correlation between number of probands with other injuries (%) 1956–1959 and different variables described in Chapter XI

CONSUMPTION OF ALCOHOL

A positive correlation was found between the number of other injuries and the consumption of spirits For group A however the difference was small and not significant

The number of offences during drunkenness was positively correlated with the number of other injuries (Figs. 70–71) It should be mentioned that no correlation was found with industrial injuries

SMOKING HABITS

The individual variables illustrating *smoking habits were not found to be* significantly correlated with absence The variables were however also taken together in the following way Group 1 consisted of non-smokers, group 2 of those who smoked cigarettes or the pipe independently of the amount of tobacco consumed and group 3 consisted of those who smoked both cigarettes and the pipe, independently of the amount of tobacco consumed With the Facit EDB which on dichotomization with the border set between group 1 and group 2 a positive correlation was found ($\chi^2 = 7.0$) A closer analysis revealed that the risk was highest for group 2 and lowest for group 1 Group B did not deviate substantially from group A The value of this correlation is limited by the fact that it did not take into account the amount of tobacco consumed

BODY-BUILD

Among the variables used for studying *body build a correlation was found only* between industrial injuries and muscle factor *shoulder thrust and subcapsular*

and parathoracic skin fold (Table 42). In all of these cases the correlation was negative. On closer inspection it was found that the correlations were not linear and that the differences in group A were fairly small. Since a correlation was found only for the muscle factor and fat factor no conclusions are warranted until the correlations have been confirmed in a larger material.

PHYSIOLOGICAL AND CLINICAL DATA

Here a correlation was found only between the number of other injuries and pulse rate at 900 kpm./min. The correlation was due to group B in which those who were in relatively good condition had most accidents. Among the 25 who could not perform the test, only one had had an accident and he belonged to group B.

DISCUSSION

The cause of accidents is at present receiving much space in the literature and accidents are often ascribed to "the human factor". A point that has received much attention in recent years is the occurrence of persons who are more liable to have accidents than others (e.g. HAGBERG 1960, FORSSMAN 1961). The present investigation included too small a number of accidents to permit any contribution to this debate. Summarizing, however, a greater correlation was found between accident and conditions at working place than with body build, habits and home environment.

A significant correlation was, however, found between accidents before and after 1956. This may be due to the individual having more risky work, but may also be interpreted as a tendency to accidents. If the material really included some people who were more inclined to have accidents than others, the risk of meeting with an accident after working hours would be greater for those who had industrial injuries than for others. As mentioned, the material included 71 individuals with industrial injuries (ii) and 30 with other injuries (oi); 14 had both industrial and other injuries. If the risk of industrial injuries and other injuries were independent, we should find P_{ii+oi} to be

$$\frac{71 \times 30}{260 \times 260} = 3.35\ \%$$

The value found was

$$\frac{14}{260} = 5.4\ \%$$

The difference is not significant, so that this material does not support the theory of some people being more inclined to meet with accidents than others. This also holds for each group separately.

SUMMARY

Different variables were studied for any correlation with industrial injuries and other injuries. In the present material the risk of accidents was greater at working places with heavy, dirty and noisy work. The material does not support the theory of some people being especially inclined to meet with accidents. The material is not large enough to warrant any conclusions concerning tendencies to accidents, and the number of accidents were studied only to elucidate any connection with sick absence.

CHAPTER XIV

EFFECT OF DIFFERENT VARIABLES ON DISTRIBUTION OF ABSENCE AMONG DIAGNOSTIC GROUPS

In Chapter 13 sick absence was treated as a whole This is correct from a point of view of the Company which has no reason to be interested in other aspects of sick absence than its duration and frequency and whether it can be expected to increase or decrease in the future As shown in Chapter 13 *it is true* that one can find correlations which appear to hold for the total sick absence but it is nevertheless necessary to distribute the absence among different diagnostic groups and in some cases also among separate diagnoses In this chapter *an attempt was therefore made to* assess the effect of different variables on the distribution in diagnostic groups of diseases. The different groups were, however very small so that only a few of the differences found were statistically significant. It was therefore not con*sidered worthwhile studying anything* but the difference between group A and group B and how the four body build variables according to LINDEGÅRD influenced the frequency distribution of diseases in group A

Two types of distributions were used. one *described by* LINDGREN *(195?)* which showed the number of days of absence for 100 employees in the course of one year and here called "absence curves and one showing the number of persons in per cent developing a given disease during the 4 year period studied *and here called* the "risk of developing a disease" In both cases as before only absence supported by a medical certi*ficate was included i.e* absence of more than 8 days duration

COMPARISON BETWEEN GROUP A AND GROUP B

(Fig 72.)

In this study group A was divided into two subgroups To A 1 were ascribed the 18 abstainers and to A 2 those who were moderate drinkers without any known conviction for drunkenness in *their history*

The statistically verified difference was found only for diseases of the respiratory tract so that the risk was lower for A 1 than for B ($p < 0.05$) Other findings of interest are accounted for below

V Mental diseases The risk was 0 in A 1 5 % in A 2 and 14 % in B The differences were more impressive in the absence curves

The test of significance between 0 14 % is meaningless

Circulatory disorders The risk in A 1 was 22 % in A 2 it was 1 % and in B it was 6 The absence curve showed a high absence for A 1 and B and a low absence for A 2 In the interpretation of this finding one may study the distribution of the individual diagnoses It was found that the high absence in A 1 was due to 3 cases of varices which together caused an absence of 170 days and one case of myocarditis which caused 25 days absence In B there were 2 workers with

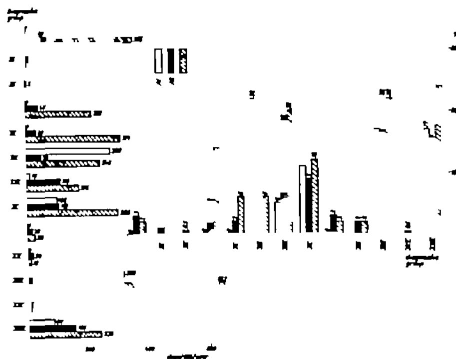


Fig. 72. *Comparison between probands according to alcoholic habits (A 1 teetotalers, A 2 moderate consumers without any known drunkenness, B consumers with drunkenness in history). Number of persons (%) with sick absence during 1956—1959 classified according to diagnostic groups and number of days per 100 probands per year classified according to diagnostic groups. See abbreviations.*

hypertension and one with myocardosis with together 496 days of absence. In A 2 there were 5 workers with varices and haemorrhoids, 2 with myocardosis and one with hypertension.

Th chances f person having myocardosis and hypertension ppears to be somewhat higher in group B

IX *Diseases f the digestive tract.* — The risk per cent was the same f the different groups, but the duration was three times longer in group B It was interesting t not that the absence in A 1 was du entirely t two operations because f hernia, while the risk f peptic ulcer in A 2 wa 4 and in B it was 22 % The 5 cases of gallstone were all f und in A 2

XIII. *Diseases f the organs of locomoti* — The risk percent is not influ enced in the variables but the durati n of absence was twice as high in B as in A 1 For diseases of the spine only the risk in A 1 was 11 % and in A 2 17 % and in B it was 14 % The duration of absence per individual and year was 3.., 2.2 and 6.6 days, respectively

XVII *Injuries.* — The risk of injuries causing absence was somewhat larger in group B and the duration was ..7 times longer for group B than for A 1

CHAPTER XIV

EFFECT OF DIFFERENT VARIABLES ON DISTRIBUTION OF ABSENCE AMONG DIAGNOSTIC GROUPS

In Chapter 13 sick absence was treated as a whole. This is correct from a point of view of the Company which has no reason to be interested in other aspects of sick absence than its duration and frequency and whether it can be expected to increase or decrease in the future. As shown in Chapter 13 it is true that one can find correlations which appear to hold for the total sick absence, but it is nevertheless necessary to distribute the absence among different diagnostic groups and in some cases also among separate diagnoses. In this chapter an attempt was therefore made to assess the effect of different variables on the distribution in diagnostic groups of diseases. The different groups were however very small so that only a few of the differences found were statistically significant. It was therefore not considered worthwhile studying anything but the difference between group A and group B and how the four body build variables according to LINDEGÅRD influenced the frequency distribution of diseases in group A.

Two types of distributions were used, one described by LINDGREN (1957) which showed the number of days of absence for 100 employees in the course of one year and here called "absence curves" and one showing the number of persons in per cent *developing a given* disease during the 4 year period studied and here called the "risk of developing a disease". In both cases, a before only absence supported by a medical certificate was included, i.e. absence of more than 8 days duration.

COMPARISON BETWEEN GROUP A AND GROUP B

(Fig. 72.)

In this study group A was divided into two subgroups. To A 1 were ascribed the 18 abstainers and to A 2 those who were moderate drinkers without any known conviction for drunkenness in their history.

The statistically verified difference was found only for diseases of the respiratory tract so that the risk was lower for A 1 than for B ($p < 0.05$). Other findings of interest are accounted for below.

V. Mental diseases. The risk was 0 in A 1, 5 % in A 2 and 14 % in B. The differences were more impressive in the absence curves.

The test of significance between 0 – 14 % *is meaningless.*

Circulatory disorders. The risk in A 1 was 22 % in A 2 it was [illegible] % and in B it was [illegible]. The absence curve showed a high absence for A 1 and B and a low absence for A 2. In the interpretation of this finding one may study the distribution of the individual diagnoses. It was found that the high absence in A 1 was due to 3 cases of varices which together caused an absence of 17[illegible] days and one case of myocardosis which caused 2[illegible] days absence. In B there were 2 workers with

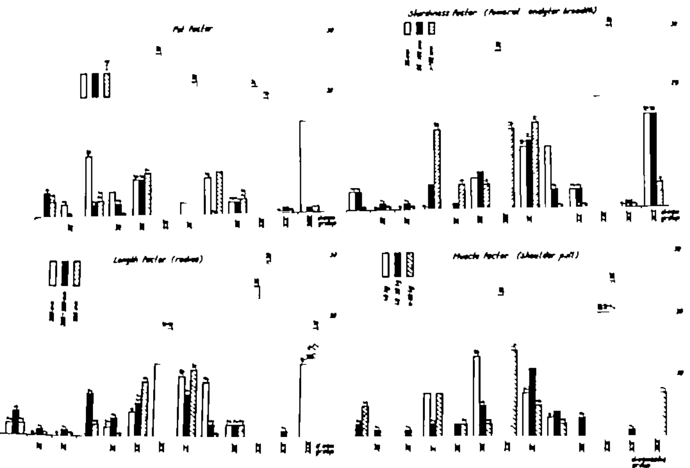


Fig. 72. *Comparison between probands with low, medium and high degree of body-build variables. Number of persons (%) with sick absence during 1956—1959 classified according to diagnostic groups. (See abbreviations.)*

FAT FACTOR

The fat factor according to Lindegard was studied (Figs 73—74)

According to Table No 42, the risk of sick absence is greater for lean individuals After elimination of group B however the curve was found to be U-shaped with "optimal health just above average body weight (Fig 66) It was therefore of interest to study the effect of the fat factor on the disease curve in group A The test for significance showed a difference in the risk of diseases of the digestive tract ($p \leq 0.01$) and for accidents ($p \leq 0.05$)

V *Mental diseases* The incidence of mental diseases and absence tended to be somewhat larger for the relatively lean workers

VII *Diseases of the circulation* did not appear to vary appreciably with the amount of body fat

VIII *Diseases of the respiratory tract* The incidence of these diseases and the absence because of them appeared to be higher for the relatively lean workers

IX *Diseases of the digestive tract* — The incidence of these diseases and the absence they caused was significantly greater for the relatively obese group The incidence of peptic ulcer was 0 for the lean 5 % for the moderately fat and 7 % for the obese and the corresponding figures for gallstone were 0 1 and 8 respectively

XIII *Diseases of the organs of locomotion* did not vary with the fat factor Nor did back weakness *per se*

XVII *Injuries* Accidents were significantly less common among the obese

The relatively lean persons thus appeared to be away from work more because of mental diseases and diseases of the respiratory tract as well as accidents while the relatively obese were away from work relatively much because of diseases of the digestive tract As for total absence because of sickness the obese differed only slightly from the lean In other words the fat factor is of less importance to industry than to the individual and to the community because of the overmortality of the obese

STURDINESS FACTOR

— In the investigation of the sturdiness factor the investigation was limited to the femoral condylar breadth (Figs 73—74) No statistically significant difference could be demonstrated

V *Mental diseases* — A low factor gave 0 % risk and a high factor 13 % risk. The difference is more impressive in the absence curve

VII *Circulatory diseases* — The risk of such diseases did not vary with the sturdiness factor neither the entire group nor the individual diagnosis *per se*

VIII *Diseases of the respiratory tract* — The incidence of these diseases varied inversely with the value of the sturdiness factor *The difference in duration was* however only slight

IX *Diseases of the digestive tract.* The incidence of these diseases varied only slightly with the value found for the sturdiness factor but the duration of absence was twice as long for those with a relatively high factor The incidence of peptic ulcer was 25 4 and 4 % and for gall stones 8 1 and 4 %

XIII *Diseases of the organs of locomotion* The risk of having diseases of the organ of locomotion was 30 % for those with a high sturdiness factor and 18 % for those with a low factor Difference was not as marked for the duration of absence For diseases of the back the corresponding figures were 1 14 and 30 % with increasing severity

XVII *Injuries* The risk of injury was 4 % for those with a high factor and 15 % for the remainder The difference in the duration of absence was marked

Some diseases appeared to be more common and others less common in

persons with a high and low sturdiness factor Therefore sturdiness facto does not appear to influence sick bsence as a whole.

MUSCLE FACTOR

The muscle factor was assessed by the shoulder pull (Figs. 73—74)

Neither here was any significant difference found.

V *Mental diseases* — The incidence of mental diseases did not vary with the muscle factor but absence was twice as high for those with a high muscle f ctor

VII *Circulatory disorders.* — The in cidence appeared to be higher f those with a low muscle factor for whom the absence was also higher Of those with relatively high muscle factor nly on had a circulatory disorder and that was thrombosis f a leg

VIII. *Diseases f the respiratory tract.* — The incidence f these diseases appeared to be higher for those with a low muscle fact

IX. *Diseases of the d gestive tract* — The incidence of these diseases did not vary with muscle factor All of the w rkers with peptic ulcer were in the intermediat group and of those with gallstones, the muscle f ctor was low in 1 and average in the remainder

XIII. *Diseases of organs of locomotion* did not vary with the muscle factor Back weakness occurred in 17 %, 11 % and 14 % of those with low medium and high muscle factor

XVII *Inj ries.* — Accidents seemed t be less common among those with a relatively high muscle facto

LENGTH FACTOR

— This fact wa measured by the length of the radius No tatistical difference could be demonstrated (Figs 73— 4)

V *Mental diseases.* No mental diseases occurred among those with a low factor and 7 % among those w th a factor of average value.

VII *Circulatory disorders* — These disorders were twice as common among those with a high factor but no difference was found in the total duration of absence Neither did the individual diagnoses sh w any clear tendency

VIII *Diseases of the respiratory tract* did not vary with the length facto

IX. *Diseases of the digesti e tract* did not vary with the length factor

XIII *Diseases f the organs f locomotion.* The disorders were least common among those of medium height and larger for the extrem groups. Back weakness was noted for 17 13 and 30 %, respectively for those with low medium, and high length factor and the number f days of absence per individual per year were 1.5 1.5 and 2.3.

XVII *The frequency of injuries* did not vary with the length fact r

SUMMARY

The distributions of the diseases were studied in two ways the number of day f absence per 100 individuals per year and the number of persons in per cent with absence during 4 year period.

The distribution of absence in different diagnostic groups were correlated with some variables, all f which were di ided according to a three grade scale

Since the material was small only three differences could be proved statistically

However the following tendencies deserve ttention.

Over-indulgence in alcohol (group B) increases the risk of absence particularly because of mental diseases, diseases of the respirat ry tract, diseases of the digestive tract, diseases of the organs of locomotion and accidents. This is in accord with the finding of LINDGREN (195?) for labourers



Fig. 4. Comparison between probands with low, medium and high degree of body build variables. Number of days per 100 persons per year classified according to diagnostic groups. (See Abbreviations.)

CHAPTER XV

CORRELATION BETWEEN CONSUMPTION OF ALCOHOL AND OTHER VARIABLES

It is clear from Chapters XIII and XIV that sick absence in group B was substantially higher than that in group A. As mentioned previously group B consisted of those graded as c, d or in the evaluation of "consumption of alcohol" (See page 125) To facilitate the understanding of this overmorbidity among those who had been convicted for drunkenness, those correlations that were found between consumption of alcohol and other variables will be accounted for first. In Chapter XIII the material was divided into two groups, A and B in order to eliminate the factor "conviction for drunkenness" which was found to be of dominant significance. This division also gave the distribution of group B among the different variables (Figs. 75—77) The significance of the differences between the degrees of variables was tested by the T test.

RESULTS

The variables found to be correlated with consumption of alcohol are accounted for in Table 45

The drinking habits in group B are elucidated by the following data. The last offence of those who had been

Table 45 *Variables found to be significantly correlated with the consumption of alcohol*

	P (less than)
Childhood environments	
Size of town or village	0.05
Parents and sibs	
If father living, present age	0.05
National service	0.01
Civil life	
Church attendance	0.02
Consumption habits	
Dietary habits	
Coffee	0.05
Milk	0.01
Alcohol	
Consumption of spirits	0.01
Years used	0.05
Tobacco	
Cigarettes	0.005
Years used	0.02
Drugs	0.001
Working conditions	
Responsibility	
Economical	0.05
For others safety	0.05
Risk of injury	0.05
Merits	
Quality	0.01
Care of equipment	0.01
Presence	0.001
Status	
Body build and heredity	
Height	0.05
Length of radius	0.01
Weight	0.01
Body face area	0.02
Relative heart volume	0.05
Hand grip	0.05
Hairiness	0.05
Pulse rate in 900 Kpm/min.	0.05
Working capacity	0.05
Evaluation of organs of locomotion	0.02
Hearing	0.05
Other absence types	
Absence with permission	0.01
Voluntary absence	0.001

Mental diseases and diseases of the respiratory tract are more common among lean persons while dieases of the digestive tract are more common among the obese

Mental diseases and diseases of the organs of locomotion were more common among men of stocky body build, while diseases of the respiratory tract were more common among those of asthenic type

Diseases of the circulation and of the respiratory tract as well as accidents were more common among men with poorly developed muscles

As for body height the risk of diseases of the organs of locomotion was possibly smallest for those of medium height and diseases of the circulation possibly less common among short persons

Though so few of the differences found in the present investigation were statistically significant it appears desirable in investigations of this type to take body build into account It is also obvious that it is not sufficient to treat sick absence as a whole without taking into consideration the diseases responsible for the absence

among those who had grown up in towns with more than 16.000 inhabitants, i.e. mainly in Västerås, 24 belong t group B Of group B, those coming from villages or small towns with 2,000—16,000 inhabitants represented 30 and those from smaller places than 2000 inhabitants 14 %

Parents and sibs

According to Table 45 the f ther ge was significantly correlated with drinking habits Fig. 7 shows that group B contained none of the probands whose fathers were between 75 and 81 ears in 19 9 Since there is no sense in testing f r ignificance with value of 0 in one of the variables, th variable was divided at 75 years of age, and then the risk of conviction fo drunkenness was significantly greater if the father age was below this limit. Other v riables concerning the parents and ibs gave no significant correlation, but to facilitate the interpretation of the correlation with the f ther s ages Fig 77 also hows the distributi n of group B mong the ges of the fathers at death, the ges f the mothers o the ages f the mothers t death, and the numbers of sibs. The same tendency was found for the mothers ages f r the fathers namely the lowest number of probands in group B in mean alues of the variable Further it was found th t the percentage of group B was highe among those whose f ther o mother had died young In ddition a U-shaped correlation was found also with the numbers of sibs so that the relatively mallest number in group B w found in families in which the proband had 1— sibs

National service — Of those who were exempted from n tional service because f poor health (C 3) the percentage in group B wa significantly higher than among those who did their national service (Health status A. 1)

Home Floor space f home — Of those living in a flat with a floo space of less than 30 m² only 10 % were in group B which was significantly lower than among those living in large flats. But when compared according to floor space per person, the correlation was U-shaped Of those who had 21—25 m² per person in the flat, 8 belonged to group B against 28 % and 22 % respectively for those who had more or less floor space

Church attendance *4 % of those who had never attended church during 19 9 belonged t group B against 13 % of those who had attended divine service on one occasion r more

Consumption habits — In addition to th correlation described in the introduction between the division int groups A and B and alcohol-consumption a correlati n was also found with other consumpti n habits Thus, in group B th number with a medium consumption of coffee and milk was higher in group B than that of those who consumed little r much coffee and milk, respectively

Tobacco Of those who reported that they smoked more than 10 cigarrettes a day 50 % were in group B and of the pipe-sm kers of those wh smoked more than 50 gm. f tobacco a week 35 % were in group B (th difference was not significant for pipe-sm kers) The number of years the probands had smoked was significantly higher in group B

Drugs Of those who reported that they had on some occasion or more often used leeping drugs sedati es or the like 4 belonged to group B

Working cond tions Members belonging t group B more frequently had work carrying less responsibility than group A from an economical point of view and concerning the risk of injuring others and themsel es their merits were as a rule judged as less good regarding quality handling of equipment and presence.

convicted for drunkenness had occurred on the average 10.8 years previously (Fig 41) It might be mentioned that sick absence was found to be independent of the time of the last offence 75 % reported that they had reduced their consumption of alcohol since 1955 and the number of offences known to the Temperance Board was on the average 2.8 (Figs 41 and 75) and for half of the probands only one.

Group B reported a higher consumption of spirits than group A (Fig 75) More than half of those who stated that their present consumption of spirits was 15 litres per year or more had on some occasion been convicted for drunkenness and the longer probands had been in the habit of consuming spirits the larger was the number belonging to group B None of the 50-year old persons in the present series who had been in the habit of consuming spirits for less than 16 years had been convicted for drunkenness (Fig 75) The actual amount of spirit consumed formerly was not noted but since 75 % reported that they had reduced their consumption of spirits it might be assumed that group B had an average higher consumption than group A also before 1955

In addition to these variables which directly elucidate the drinking habits the following variables were also found to be correlated with the consumption of alcohol

Childhood environments The percentage of persons in group B was higher for those born in town than in rural districts Closer analysis showed that



Fig 75. *Number of probands (%) convicted for drunkenness (Group B) related to consumption habits.*

among those who had grown up in towns with m re than 16 000 inhabitants i.e mainly in Västerås ...4 belong to group B Of group B those coming from villages o small towns with 2,000—16 000 inhabitants represented 30 and those from smaller places than 2000 inhabitants 14 %

Parents and sibs

According t Table 45 the father s age was significantly correlated with drinking habits Fig 77 sh ws that group B contained none of the probands whose fathers were between 5 and 81 years in 19 9 Since there is no sense in testing for significance with value of 0 in one f the variables, the variable was divided at 75 years of age, and then the risk of conviction fo drunkenness was significantly greater if the f ther age was below this limit Other variables concerning the parent and sibs gave n significant correlation, but to facilitat the interpretation of the correlation with the father s ages Fig 77 also hows the distribution of gro p B among the ages of the fathers t death, the ages f the mothers r the ges of the mothers at death, and the numbers f ibs The same tendency was fou d for the mothers ages a f r th fathers namely the lowest number of proband in group B m mean alues of the variable. Further it wa f und that the percentage f group B w higher mong those whose father r mother had died young In ddition a L-shaped correlation w found also with the numbers f sibs so that th relati el mallest n mber in group B was found in f milies in which the proband had 1— ibs.

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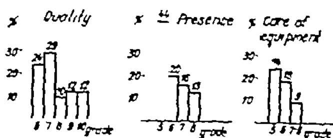
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Working conditions Members belonging to group B more frequently had w rk carrying less responsibility than group A from an economical point of view and concerning the risk f injuring others and themselves, their merits were as a rule judged as less good regarding quality handling of equipment and presence



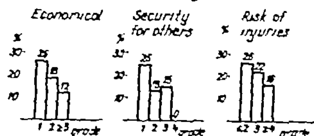


Fig. 6. Number of probands (%) convicted for drunkenness (Group B) related to conditions at working place.

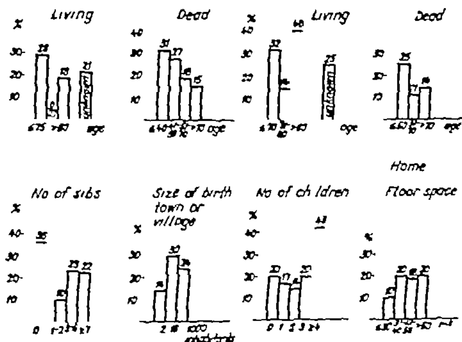


Fig. — Number of probands (%) convicted for drunkenness (Group B) related to childhood and home environments (See page 00)

Status. Body build. Stature, length of radius, area of body surface and hand-grip were all relatively low for members of group B. Body weight and relative heart volume showed a U-shaped correlation with the lowest number of probands in group B in the mean value. Finally the number with a high degree of hairiness was found to be significantly lower in group B.

Pulse at 900 kpm./min. Of those who could not perform the test, significantly more belonged to group B than to those who had a pulse below 150.

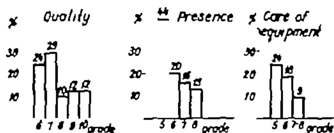
Evaluation of the organs of locomotion gave lower values for group B and of those with impairment of hearing relatively many belonged to group B. Working capacity at the time of the examination was judged as less good for group B.

Finally there was not only a correlation between alcohol consumption and sick absence, for group B also showed a definitely higher value for "absence with permission" and "voluntary absenteeism".

DISCUSSION

As mentioned, group B contained those who had had convictions for drunkenness known to the Temperance Board or to the Company and group A consisted of the remainder. It is clear from page 144 that in group B the members consumed more alcohol per year and had been drinking for more years than those in group A. That the higher consumption implies a greater risk of intoxication and conviction for drunkenness is obvious but it is also possible that the effect of a given dose of alcohol varies from individual to individual (Goldberg 1943). It is therefore not *a priori* certain whether the results obtained can be compared with those of earlier investigations of the aetiology of alcoholism and alcoholic abuse or the development of different alcoholic habits. It is therefore important to underline the fact that group B contained only 2—3 probands who could be classified as alcoholics according to the definition of WHO (see page 87). With Jellineks nomenclature they would have been graded as alpha-alcoholics. It is also doubtful whether it is justified to classify the other members in group B as abusers. Classified according to the system used by the Temperance Board, in 1944 in which only offences during the previous 10 years are taken into account, 21 of the 50 in group B would be classified as moderate consumers. Group B is thus not synonymous with abusers or alcoholics, and it is not a really uniform group concerning the consumption of alcohol. With these reservations it might however nevertheless be justified to study to what extent the correlation of possibly aetiological significance and described above agree with those found by previous authors. Jellinek (1960) showed the different alcoholic habits in different countries essentially influence the frequency and symptoms of alcoholism, and in Sweden the Temperance Board has shown that the different alcoholic habits in different parts of the country and in different social groups influence the frequency of abusers. Åmark (1951) found signs suggestive of hereditary factors, at least in periodic alcoholics. He also found that sibs and fathers of alcoholics often had psychogenic psychosis and psychopathy which might indirectly argue for a hereditary factor. On the other hand, he showed that the environmental condition during childhood and occupational factors are of importance. Kaij (1960) in his comparison between the drinking habits of homozygotic and heterozygotic twins also found closer similarities in alcoholic consumption between the homozygotic twins than for the heterozygotic, which also supports the assumption that

Merits



Responsibility

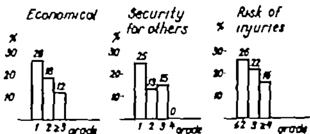


Fig. 76 Number of probands (%) convicted for drunkenness (Group B) related to conditions at working place.

Father

Mother



Home

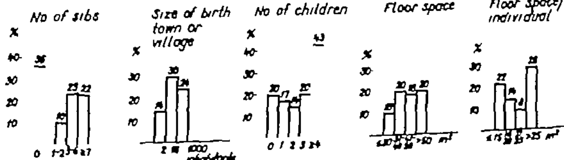


Fig. 77 Number of probands (%) convicted for drunkenness (Group B) related to childhood and home environments (See page 00)

heredity plays a dominant role in the development f habits.

It is clear from Table 45 that there are large number f variables howing a significant correlation with the distribution of the material in groups A and B. Of these correlations, however only some can be regarded as being of aetiological significance. This does n t apply for example to the conditions at the present working place It has been found that the merits for quality quantity and presence were lower for B and that B members oft n had work with less responsibility This also holds for the variables f consumption ther than alcohol as w ll as rel gious habits and absence with permission and voluntary absenteeism. These variables supplement the characterizati n of group B but cannot be of aetiological significance.

The correlation with the size of the present flat and floo space per individual can be explained by the married probands in group B having m re children than those in group A and the tendency f an increased risk f belonging t group B if th probands were unmarried t the time of examination.

Group B contained relati ly many persons exempted from military service on grounds f health This might be interpreted in different ways g that those wh were C-3 but were degraded experienced their exemption as a psychical tra ma, which increased their consumption f spirit and resulted in conviction f drunkenness or that the properties which sooner or later resulted in drunkenne had also infl enced their health already at 0 years. The present investigation does not permit any st tement concerning the probability f either of these or ther theories.

Sick s. — As ment oned on page 130 ick absence was found t be correlated with some ariables which were the results f linical in estigation g w king capacity evalu tion of rgans of locomotion and pulse rate in 900 kpm./mm. Since group B has a high sick absence it is but natural that it should deviate from group A also regarding these variables. This will not explain why group B had significantly higher value f impairment of hearing It is not clear how this correlation should be interpreted. At the time of the examina tion the members of group B were n t exposed to greater noise t their working places than group A, but nothing is known about the noise they had been exposed to previously Neither is it known to what extent the probands took precautions t protect themselves against the noise. Thus, the results obtained in the present investigation d warrant any conclusion as to whether a high consumption of alcohol increases the risk f impairment f hearing on exposure t noise

Body-build and heredity Group B comprised many members of short sta ture and with poorly developed muscles (Fig 78) Why this variable hould vary with the consumption of alcohol is not known with certainty One might imagine that the comparati ely short probands with poorly developed muscles in this material had used more alcohol to trengthen their self-assurance, parti cularly since LINDEGÅRD & NYMAN (1956) found a correlation between body build and personality described by Sjöbring radicals (LINDEGÅRD, however found n correlation between body build and personality when the personality was studied by questionnaires 196_)

Another possible explanation is that relati ly small persons cannot tolerate alcohol so w ll those of greater stature Another point that has appa ently not been discussed previously i whether hairiness f the chest i correlated with the consumption f alcohol. It is clear from Table 46 that the degree of hairiness varies with ther body build variables so that thi may explain the

Body height

Body weight

Body surface area

Relative heart volume

Length of radius

Hand grip

Hairiness

Capacity working

Organs of locomotion

Loss of hearing

Pulse rate in 900 kpm/min

Fig 8. Number of probands (%) convicted for drunkenness (Group B) rel ted o body build, heredity d status.

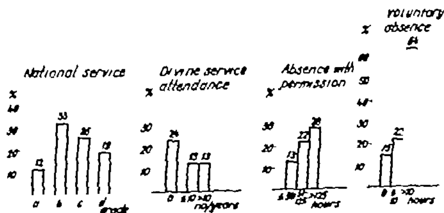


Fig. 79 Number of probands ( ) convicted for drunkenness (Group II) related to other variables

SUMMARY

Chapter XV gives an account f the variables that proved t be significantly correlated with the distribution of the probands in groups A and B i.e correlation with consumption f alcohol. Of all of those correlations ccounted fo in Table 45 only the variables reflecting childhood environment par ents and sibs, body build and hair growth can presumably be of aetiological signif icance f this distribution.

correlation with alcoholic habits. LINDEGÅRD & NYMAN also found a correlation between hair on the trunk and Sjöbring's variable "stability" (LINDEGÅRD 1962)

Childhood environments and parents and sibs The size of the village or town where the probands had grown up was found to be correlated with consumption of alcohol (page 144) This correlation may be explained in various ways

1 According to the report of the Temperance Board 1944 states that the consumption in the country was lower than in the towns ÅMARK (1951) found that alcoholics more frequently came from homes where the brothers consumed 3—8 times more alcohol than the average amount ALLARDT (1957) showed that the habits are influenced by the group the individual belongs to It is therefore likely that those who grow up in the country have different drinking habits from those in town

2 VAN ALPHEN DE VEER (1955) showed that later success or failure in life depends largely on the contact with the parents and particularly with the father According to ÅMARK (1951) the risk of alcoholism is greatest among those coming from broken homes. Applied to the present material it appears possible that the contact between the parents and the children in the country was better when both parents worked near the home and the children have the possibility of observing their parents also during their daily work In the town children have much less opportunity of meeting at least the father during week days If VAN ALPHEN DE VEER's theory is correct children growing up in the country have a better chance of succeeding in life their alcohol requirements will be less and the number of convictions for drunkenness lower

Are body build and childhood environments independent? With the Facit EDB it has been possible to examine to what extent the variables for body build and hereditary were correlated with environmental factors It was found that the size of the place where the probands grew up was significantly negatively correlated with the length of the radius χ^2 10.36, $P < 0.01$ bimalleolar breadth χ^2 4 0 $P < 0.05$ muscular strength best hand χ^2 4 7 $P < 0.05$ shoulder thrust χ^2 4.8 $P < 0.05$ and shoulder pull χ^2 11 4 $P < 0.001$

That body build is not independent of the size of the town in which they are brought up in the present material may appear surprising but the following explanation may be tentatively offered

1 It is only part of the male population that go into industry or take up a trade It therefore appears probable that that part of the population of the town that take work in the town they are living differ in composition from that part of the rural population that seek work relatively far from their home towns

2 When those probands in the present material grew up during and after the first World War it was probably often difficult to obtain sufficient milk or other protein for the working cla children in the towns The possibility was probably greater in the country also for the children of poor farm labourers This might have influenced the growth of the skeleton and musculature

It is not necessary to decide which of these theories is the more probable It is sufficient to state that the correlation between the environmental factor size of village or town in which they grew up and body build appears reasonable

2. Morbidity is a direct consequence of the alcoholic intoxication during the last few years. Neither did this appear probable. It is true that one cannot rely completely on what the probands stated concerning their consumption of spirits, but the last convi tion for drunkenness was on the average more than 10 years previously since the time of the last conviction for drunkenness was not correlated with sick absence

3 The body build or childhood environments r both in combination influenced not nly drinking habits or tolerance t alcohol, but also resulted in group B being more sensitive and anxious in the event f illness so that they were on the sick list f r a longer time than group A

4 F the same reasons as those given under 3 the resistance to diseases is decreased w th low t lerance to alcohol r with increased consumption.

This theory would agree with the conception that th resistance of problem drinkers t psychosomatic diseases is decreased (e g MIRSKY 19 ") or that the stability of the vegetative nervous system is lowered (MINDUS 1956)

If theory 3 r 4 were correct, those in group A with the same characteristic grades f variables as those found to increase the risk for group B would ha higher sick absence B t this was n t found t be the case

5 According t SELYE 1946 the "general adaptation syndrome" may occur after intoxication. One might imagine that group B were in the "stage of resist nce" after acquired adaptation to alcohol. This stage is characterised by increased resistance to the pecific agents (in this case alcohol) and decreased resistance to other types f tress The adaptation would explain why the average interval since the last con viction for drunkenness was so long The intoxication is supplemented in group B also by relatively high consumption f tobacco and of drugs Not all diseases can be explained by SELYE s theory but it ppears that it probably can in the diagnostic groups in which group B showed overmorbidity respiratory tract infections, back and joint sympt ms, and peptic ulcers

That part f group A with the sam characteristics as group B had, as men tioned, lower sick absence than group B The only difference that could be demonstrated in the present material was, in addition t conviction f alco hol in group B, that group B had used alcohol for a longer period than group A. It would thus appear that the greater sick absence f those 50 year old wor kers wh had some time in life been convicted f drunkenness could be ex plained t least in part by a higher consumption f alcohol for a longer peri od than those in the rest of th material.

CHAPTER XVI

WHY HAVE PERSONS CONVICTED FOR DRUNKENNESS HIGH SICK ABSENCE?

The high sick absence in group B (individuals convicted for drunkenness) which if allowance be made for time was 3 1 times higher (page 125) than absence in group A ($p < 0\ 001$) is in good agreement with earlier investigations

But in the previous investigation the individuals studied were as a rule heavier drinkers than in the present material. THORPE et al. (1959) for example reported 2.5—3 times a higher sick absence for alcoholism MAXWELL (1959) found the ratio to be 2.5 1 for problem drinkers, and LINDGREN (1957) who studied individuals who had been reprimanded because of drunkenness at work, found twice as high a frequency of sick absence.

The fact that the distribution in groups A and B (Chapter XV) were found to be correlated with other variables (Table 45 page 143) does not explain why group B has a higher sick absence than group A.

In Table 42 a correlation was found only with certain variables in the status, namely working capacity and evaluation of organs of locomotion with other absence

The correlation with status showed that sick absence in group B can be regarded mainly as disability because of poor health, and the correlation with other absence that group B also had a high tendency to absence

It was therefore investigated whether any combination of variables was responsible for this increased absence For this purpose variables were combined according to Table 45 so that childhood environments and father s age were taken together habits other than drinking habits were taken in one group working conditions in one etc. It was then examined whether sick absence in group A was influenced by these combinations of variables Various combinations were also made between childhood environments on one hand, and body build and herdity on the other In none of these cases was any increase of sick absence in group A found for that part in group A that had those values for the variables characteristic of group B

The high sick absence in group B must thus be explained in another way

The following theories may be suggested

1 Sick absence is only a name for absence due to acute alcoholic intoxication This theory has found support in the literature and in general but it is supported only by STEVENSON s investigation from a mine in 1940 LINDGREN like MAXWELL and THORPE, has clearly shown that this theory is not probable Neither did the present investigation provide any evidence in support of this theory The main part of the absence was verified by *medical certificate and* it was not particularly the short time absence that was influenced

2. Morbidity is a direct consequence of the alcoholic intoxication during the last few years. Neither did this appear probable. It is true that one cannot rely complet ly on what the probands stated concerning their consumption of spirits, but the last con iction for drunkenness was on the average more than 10 years previously since the time of the last conviction for drunkenness was n t correlated with sick absence

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the males while the number of spells of absence was influenced only slightly by age. Among the females the absence was greatest for those in middle age, i.e. that period of life when they have most work to do in the home because of their children or ld relatives. It was also found that the salaried part-time female employees were way from work less than full-time employees of the same age.

In the course of the investigation it proved necessary to study the importance of certain statistical sources of error (Part II) Part II deals with the influence of the number of waiting days of the General Sickness Insurance on the distribution of sick absence among diagnostic groups. It was found that when absences of bort duration were included, diseases of the respirat ry tract and to som extent also f diseases of the digestive tract and organs of locomotion in the mal salaried em ployees assumed greater importance as cause of absence. In other words, it was particularly in these diagnostic groups that absence of bort duration occurred. It was also found that the records of the General Sickness Insurance are incomplete and d not give a complete picture f the working disability because f disease This is due above all to those members of the sick insurance wh are not forced by thei employers to report t the insurance when they are absent because of disease

It is regarded as self-evident that if the number of working hours ffered is hort the average sick bsence will also be short. It is therefore usual in such investigations as the present one t correct absence according to the tim f employment f r those wh have not been employed through ut the time covered by the statistical analy is It is, however lea from Part II that the proced re is not theoretically satisfactory because the period prevalence rat for those who ha been employed f r only a short time was significantly lower than it should be if it were directly proportional to the duration of employ ment, and that the number f industrial injuries is not directly proportional to the duration f employment but is also influenced by the time the individual has been employed. In the investigation of absence the material should preferably consist f employees who have been employed during the entire period covered by the investigation and comparisons should preferably be made only between series covering an equal observ ation period

Mortality tatistics are usually given as a measure of the state of health. It was therefore interesting to note that in Part II the mortality was not found to influence the sick absence statistics.

Finally in Part II analysis by various methods showed that the distribution f absence among the employees cannot be ascribed to chance It was theref re considered legitimat to extend the work in Part III and to investigate what f t rs might possibly infl ence sick absence.

F r this purpose all Swedish male workers that wer employed in the Company throughout th period covered by the in estigation and born in Sweden between the years of 1909—1913 were studied. It was assumed that since sick absence is not due t chance it must be due to hereditary or acquired fact rs or to environmental conditions. A large number of variables were theref re studied. Dat were btained partly from available registers *within or outside the* Company partly from personal inter views cond cted by the utho and inf rmation on the working conditions was supplemented by personal inspection of the working places and on the physiological tatus by examination of body build and clinical examination. All of these variables were then correlated

CHAPTER XVII

GENERAL SUMMARY

Absence from work because of disease means a considerable economical loss not only to the individual, but also to his employer and to the community in general. As pointed out by FORSSMAN for example sick absence is above optimal level, for which reason it is desirable to chart the diseases responsible and to find means of improving the general state of health and reducing sick absence.

It was with this in mind that the author undertook an investigation of the absence in a relatively large Swedish company (ASEA, Västerås) which had 9000 employees in 1959 To provide a background to the material studied, a brief description is first given of the environments of the employees during childhood and of their present home conditions and working conditions Both the town of Västerås and the Company are at present rapidly expanding and many of the employees come from places outside Västerås As many as 20 % of the employees were born abroad i.e roughly as many as those who were born in Västerås Owing to the rapid expansion of the town the houses are largely modern. As in many expanding industries in Sweden, the housing problem has for many years made it difficult to find satisfactory dwellings for employees from outside Västerås The working conditions largely fill modern requirements

The norms suggested by the International Conference on Sick Absence have been used in the description of absence of the employees It was above all the number of spells of absence (the period prevalence rate) and the duration of absence (the average duration of completed or incompleted spells observed per person under observation) that were studied. In addition, sick absence was classified in diagnostic groups according to the disease nomenclature of WHO The difference in absence with sex and age was studied and compared for salaried employees and workers. In addition, the absence in the Company was compared with literature data and with absence in other Swedish companies

Despite the size of the company the absence was not greater but if anything possibly less, than that in other companies studied.

Among the workers the diseases responsible for the largest number of days of absence were: diseases of the skeleton and organs of locomotion, of the respiratory tract, mental diseases and diseases of the digestive tract and injuries Among the salaried employees diseases of the bones and joints were of less importance The diseases responsible for most of the absence, particularly for absences of short duration of the salaried employees were infections of the respiratory tract.

Women were away from work twice as much as men and the workers twice as much as salaried employees. The difference between the workers and the salaried employees was not due to the number of spells of absence but to the duration of absence and was ascribable particularly to low back pain, diseases of the digestive tract and of the respiratory tract The duration of sick absence increased with increasing age in

Comparison between group A and group B *showed that group* B had a highe absence, particularly f r diseases f the back, but also f r the groups injuries mental diseases, diseases of the respira tory tract and of the digestiv tra t.

In addition, the influence of body build was studied afte distribution *mong the diagnostic groups* It was found that the four different body build factors according to LINDEGÅRD gave a correlation with different diseases in low and high grad of variable When absence because of the different diseases was added, the influence of these ari ables on absence therefore *disappeared.*

It is clear from what was said above that the sick absence in group B was much higher than that in group A. It was theref re investigated which vari ables decided whether a person wa signed t A or B It was found that members in group B had, in ddition t con iction f drunkenness probably consumed a larger average mount of lcohol and f larger number f years than the members f group A. In addition, a numbe of correlati ns were found between several variables and group A and B respectively It appeared, however that the only variables of possible etiological significance in the assignment of a person to group A or B were childhood environments and par ent and sibs, on one hand, and body build and heredity on the other On an attempt to judge whethe it was *heredity* or childhood environment that was f greater importance it was found that these two variables were not independent of one another

Finally an attempt was made to find out why members of group B were w y from work m re than those f group A. This question could n t be answered with certainty The properties characterising group B had no influence on sick bsence among those wh had the same properties in group A. Neither wa it possible to show that combination f these properties increased the side absence in group A The only respect in which group A wa found to diff from group B was in addition to con ection f drunkenness that th members f group B had probably consumed relati ely more alcohol than members f group A.

Juding from Part I where it w found that th relativel high ick bsen ce was due t th severity rate nd n t t th frequency rat and that it was th organs of locomotion that were t large extent responsible for sick absence it ho ld be pos ible t reduce ick absence among the w rkers the elaboration f ergonomical method f eliit ting the w rk and improvaing th working con ditions in general.

The investigation also ggested that absence mong the females could probably be *reduced by dju ting the* wor king hours ccording t the household and social duties of the w men. Absence among the females, however requires f rther investigation on a larger series bef re any conclusions can be drawn

The group of w kers in P rt III were unf rm in various respect and were characterized by relatively good dapat tion t thei work This might h lp t explain why a correlation could be found betwen sick absence and con ditions t th w rking places It might also explain why no correlations were found between absence and ther fac tors either

This does not limit th value of the positive fi ding that sick absence wa strongly correlated with the consumption of lcohol. Since th investig tion also h wed that the drinking habit varied with th en ironments during childhood, it would ppear plausible to sume that ick absence in middle age can be reduced by the creation f s ch hild

with sick absence (excluding accidents) industrial injuries and other injuries calculated as the number of spells and the number of hours of absence

With the aid of a data machine mean values and standard deviations of the variables were calculated Since it was found that only few of the many variables studied showed a normal distribution, the mean value and standard deviation did not provide sufficient information, therefore the values found for the variables are also given graphically

With the aid of the data machine all these variables were afterwards correlated with sick absence, excluding industrial and other injuries The variables were then correlated with the number of spells and the number of hours of absence within the observation period of time. In the calculation use was made of χ^2 after dichotomization of the variables. This gave a good impression of the probable correlations, but since the distribution of the material was generally not normal for the variables, it was necessary to analyse the correlation further It was also found that the variable "number of convictions for drunkenness" was of such dominant significance that it influenced the correlation between other variables and sick absence The probands were therefore divided into two subgroups group A and group B according to the variable "evaluation of drinking habits" The total abstainers or those who consumed only moderate amounts of alcohol without conviction for drunkenness were assigned to group A and the remainder i.e those who had on some occasion or other been convicted for drunkenness to group B The next step was to calculate the correlation, in group A between absence excluding injuries and all other variables The mean values for absence were calculated for the different degrees of variables and the differences between the different degrees were tested for significance by pairs with the t test The result showed that the correlations found fell into three groups (1) obvious correlations with variables which because of their nature must be correlated with the sick absence (2) correlations with other forms of absence absence with permission and overtime and (3) correlation with two variables from the merit score at the working place namely the factors quality and quantity The correlation found between quantity and sick absence was expected Since a strong correlation was found between the different merit variables this also explains the correlation with quality

In group A it was thus not possible to demonstrate any variable that could reasonably be supposed to be of significance as a cause of sick absence excluding injuries

Although the material was less suitable as a basis for the discussion of industrial and other injuries these absences because of injuries were nevertheless correlated with other variables by the χ^2 test It was found that the risk of industrial injuries in the present material was greater among workers occupied with relatively heavy noisy and dirty work No evidence was produced in support of the belief that certain people are more predisposed to injury than others

Since sick absence cannot be regarded as a simple factor but a a composite factor i.e the sum of absence because of different diseases interest was also focused on the influence of different variables on the distribution of absence in the diagnostic groups The number of individuals with diseases in the different diagnostic groups after this classification was small, so that the significance at the 5 % level was possible in only few instances Certain tendencies were found however which deserve mentioning

ACKNOWLEDGEMENTS

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The choice of methods for st tistical analysis was made by Fl. l Björn Höljer who together with Eng Per Gjerlöv of the Department of Numerical Analysis, ASEA programmed the data-machine and who, with the aid f the dat machine tested the differences for significance

The Department f Ind trial Hygiene of Swedish Employers Federation placed their extensi e xperience t my disposal as well a thei library particularly for the st dy f absence

After ha ing btained pecial per mission the Local Temperance Board and the Local General Sickness In rance placed their regi ters at my disposal. Dr Sundberg, hospital denti t, recommended the variables of the dental status suitable for the present investigation.

The management of ASEA and col leagues at the Health Center of ASEA facilitated the work in every possible way It was the late Directo Sven Erik Eriksson who paved the way for the present investigation and thereby once more showed his great interest in the welfare of the employees and particularly in their health.

The local branch of the trade unions actively supported the investigation and the individual probands invited t take part in the investigation cooperated willingly

Many other persons in the employ of ASEA ha e assisted in the investigation. The department for measuring technique calibrated all the measuring instruments used Engineer K. Hernod f the timemotion department took part in the st dy of the workingplaces, Engineer B Olsson was responsible f r the mea surements of noises, Doctor Rune Raxell performed heart olume measurements; Nurse Edith Juhlin, the audiometric determinations Nurse Göta Andersson the functional and anthropological me surements and the copy department, copies of the figures

In the course of th work valuable suggestions were ffered by my present chief Dr Allan Hedfelt, by my former t acher of internal medicine D H Silwer and by many others

I t ke this opportunity of thanking all those who have in some respect or another contributed to the present in estig tion.

hood environments as do not encourage the later use or abuse of alcohol

Registration of all absence is desirable in all Swedish industries, the industries the investigation having shown that such registration is a god measure of the health of the workers It would probably also provide a good tool for estimating the general health of the working population.

evaluation systems vary both in n menclature and number but in all systems factors can be classified under four main factors, namely *skill*, *responsibility* *exertion*, and *conditions at working place*

These four main factors are mel ded in ASEA s work evaluation system The main factors are divided into a total of eval ation factors, by which the work is judged. Each evaluation factor is defined and divided into fi e grades from one t f ve Each grade has definite tandard.

In order to btain a proper balance between the evaluation factors extensive analyses and practical evaluations were perf rmed

EXAMPLE OF REQUIREMENTS OF WORK

EVALUATION FACTOR THEORY

Definition The fact r refers to the demands of the job on knowledge that can be acquired by theoretical studies The evaluation of the requirements should not be influenced by the kn wledge of persons now doing th job

Remarks. This fact r includes all theoretical knowledge that can be acquired by attending courses and schools E en if some of the knowledge can be acquired by practical work, it hould nevertheless be regarded a "theoretical knowledge"

Grade	Description	Points
1	Requiring elementary school knowledge	0
2	Requiring evening course for one term	1
3	Requiring 1—2 years evening courses at technical school	2—3
4	Requiring knowledge corresponding to 3 years course t industrial school	4—6
5	Requiring much more knowledge than that given under 3	7

Practice.

Defi ition This factor refers to the practical experience expressed in terms of the time normally necessary for learning th job The necessary time includes that necessary for practical training t an occupational o in dustrial school and included under the heading "theory"

Grade	Definition of grade		Points
	— 1 month	0	0—1
	1— 3 months	1	
			2—4
	6— 9	3	
	10—12	4	
3	1— 2 years	5	5—7
	2— 3	6	
	3— 4	7	
4	4— 5 years	8	8—11
	5— 6	9	
	6— 7	10	
	7— 8		
5	8 years		12

APPENDIX

EXTRACT OF DESCRIPTION OF EVALUATION OF WORK IN MANUAL OCCUPATIONS

GENERAL

The price of most goods depends upon quantity type and general price level This also applies to the pricing of service i.e determination of wages for a given piece of work. Primitive procedures formerly used for determining the prices of goods have been superseded by more exact measuring methods. This development also holds for determination of wages. With properly performed time-motion studies it is now possible to make determinations of quantity i.e a determination of the amount of work. And some 20 years ago U.S.A. introduced methods for evaluating types of work This provides a tool facilitating appraisal of the relative value of different kinds of work. Time-motion studies and evaluation of work thus provide a possibility of measuring two of the three components determining wages namely amount of work and the nature of the work. The third component, the price level of wage level varies with the state of the market, the site of the factory and, to a certain extent with the availability of labour The wage level is therefore a question settled by agreement between employers and the employees When deciding wages, the amount of work and the nature of the work should be studied by time motion studies and evaluation of the work, respectively while the general level of the wages is determined by agreement between the parties concerned

PURPOSE OF EVALUATION

The purposes of the evaluation of work are

1 to establish correct relationships between the wages for different types of work in a given company
2 to obtain a concrete basis for discussion of the level of wages in the company and
3 to create possibilities for a wider variation of wages in order to stimulate professional or occupational training also for relatively low rank occupations
4 to insure a more even distribution of recruits for those occupation occurring in the company

CONSTRUCTION OF SYSTEM FOR EVALUATING WORK

The various jobs are evaluated with the aid of a work evaluation system The most common type is the so-called point system The evaluation system applied at ASEA and devised in association with representatives of Sveriges Verkstadsförening Svenska Metallindustriarbetarförbundet and Svenska Gjutareförbundet is designed according to this principle An evaluation according to this system aims at assessing the degree of difficulties of a job and the conditions under which it is performed with respect to certain definite factors

The factors occurring in the point

Economical responsibility

Definition This factor refers to the attention and sense of responsibility to avoid economical loss and loss of good will of the Company. Loss may be due to damage to, or loss of tools, equipment, material, continued working of unacceptable parts, finished products, tools, etc.

Remarks: In the evaluation of this factor only the risk and order of losses are taken into account. Only the risk of damaging the Company's good will is evaluated because it is not possible to assess the extent of such damage.

Grade	Definition of grade	Points
1	No or insignificant losses	0–1
2	Risk of definite but relatively small economic losses.	2–4
3	Risk of larger economic losses, or risk of smaller economic losses and small risk of damaging the Company's good will.	5–7
4	Risk of very large economic losses, or risk of damaging the Company's good will or risk of large economic losses and small risk of damaging the good will of the Company	8–11
5	Risk of heavy economic losses and risk of damaging the good will of the Company	12

Responsibility for others' safety

Definition The factor refers to the demands on care and observance of established precautions to avoid injury to other persons. If two or more persons perform a job together and all must observe the same precautions, the work of each should be evaluated with respect to the responsibility carried by the job. If there is a risk of injurying several persons the number of points is increased by one.

Remarks In the evaluation of this factor both the risks and the order of such risks are taken into account.

Grade	Definition of grade	Point
1	Work incurring no or small risks of damaging others.	0
2	Work which requires attention for avoiding minor reversible injuries e.g. cuts, burns, etc.	1
3	Work requiring care for avoiding minor injuries.	2–3
4	Work sometimes requiring care to avoid serious injuries.	4–6
5	Others' safety depending entirely on the behaviour of the person performing the work in hand and requiring continuous attention to avoid serious damage	7

Sense of judgement and initiative

Definition This factor refers to the demands of the job on sense of judgement and initiative to cope with new situations, to plan and perform work independently and to choose the best method for the work to be done

Remarks This factor also takes into account any effect of the evaluation of the factor "experience". This applies above all to work requiring *long practice because of the necessity* of learning certain complicated but limited tasks

Grade	Definition of grade	Points
1	Work performed in accordance with detailed oral or written instructions. Problems arising are, as a rule decided by the foreman or supervisor	1
2	Work performed independently *but in accordance with in*structions and a certain routine. Changes in routine may be necessary	2—4
3	Independent work requiring decisions on questions con*cerning the planning* and performance of the work etc	5—7
4	Independent work placing high demands on sense of judgement and initiative.	8—11
5	Independent work placing high demands on initiative and sense of judgement without consultation of supervisors.	12

Skill and dexterity

Definition This factor refers to the demands on inherited and acquired properties resulting in rapidity of reactions and accuracy of movements *e g* established tolerances sense of order etc.

Remarks The description of the grade includes equal demands on skill and dexterity If a work places different demands on the worker in this respects the number of points should be adjusted accordingly

Grade	Definition of grade	Point
1	Work requiring little skill and dexterity	0—1
2	Work requiring coordination of movements and certain degree of accuracy that can readily be learned.	—4
3	Work requiring acquired skill for a limited number of steps of *a job and requiring care to* obtain a certain degree of accuracy	5—
4	Work requiring acquired skill for a number of different steps and care for obtaining the necessary accuracy e.g. established tolerances in association with work on machines.	8—11
5	Work requiring skill for the performance of a number of different steps and great care for obtaining necessary accuracy e.g. narrow tolerances of hand made parts.	1

Psychical stamina.

Definition This facto ref rs t the mental strain of the monotonous work in a given posture

Remarks Here posture is to be under stood as a posture occupied by people with stationary work.

Grade	Definition of grade	Points
1	Alternating jobs in different working places.	0
2	Alternating jobs at same working place	1
3	Sometimes monotonous work	
4	Monotonous or work partly at same working place.	3
5	Very monotonous or monotonous work at one and the same working place.	4–5

Environments

Definition This f ctor refers t the general working conditions (temperature draught, noise, light, dirt hemical substances dampness team dust, moke gas, smell) t the working place

Grade	Definition of grade	Point
1	Clean or only slightly dirty orking places.	0–1
	Working places not quit so clean as that under grade 1	2–4
3	Working place affected severely by one or to certain degree by several, of the above-mentioned factors.	5–8
4	Working place affected by several of the above-mentioned factors and severely by at least one	9–13
5	Working place affected severely by several of the bove-mentioned factors.	14–18

Risks of i jury

Definition The facto refers to the demand n care t avoid personal injury

Grade	Definition of grade	Points
1	Work where ordinary care is sufficient to avoid injury	0
2	Work occasionally requiring attention to avoid minor injuries such as cuts, burns without permanent sequalae.	1
3	Work continuously requiring care to old minor injuries.	2–3
4	Work occasionally requiring care to avoid severe injuries.	4–6
5	Work requiring care to old serious injuries.	7

DESCRIPTION OF WORK

Th evaluation of work is based on a careful analysis of the work t be evaluated. All conditions t be con sidered in the evaluation are accounted for in the so-called "descriptions of th work" It is, of course, important that all descriptions of th work are made according t definit principles and theref re special forms for this purpose have been worked out. In order to obtain a uniform method f description of the work uch forms should be devised by as small a number of persons a possibl The collection f data f r the different jobs can be made by different persons but all djustments of the descriptions hould be performed by one and the same person r committee

Description f a given type of w rk hould incl de all data necessary for evalu ting the work. The description of the w rk should be clear and concise A photograph or a drawing of the working place often facilitates evaluation. In this conjunction it should be underlined that the description of the work hould n t contain any assessment of the factors

Leadership

Definition This factor refers to the *demands placed on leadership* It may embrace *e g* directions for and control of the performance of the work regarding methods used, accuracy and instructions regarding order

Remarks This factor evaluates the work of leaders and includes for example, adjustment of machines and supervision or leadership The points allotted for inspection of work depend upon the extent to which the inspector is *in contact with other workers* for instruction and discussion of the quality of the work.

Grade	Definition f grade	Poi ts
1	No limited f nctions. Work sometimes requiring simpl instruction to worker are given 1 point	0–1
	Work requiring lead rship for 1 worker or work sometimes requiring leadership of a small number of w kers (2–5).	2–4
3	Work requiring leadership f mall numbe of workers.	5–7
4	Work requiring leadership of larger number of w kers (5)	8–11
5	Work requiring extensive leadership of large number of workers.	1

Physical stamina

Definition The factor refers to the *demands placed on physical stamina* *e g* moving and handling of heavy articles The frequency with which such heavy articles must be moved decides the number of points. Handling is to be understood as manual handling of material, details, tools and levers for example

Grade	Definition of grade	Points
1	Small demands on physical stamina	0–1
2	*Small to moderate demands* in the form of handling of light articles et alternately sitting and standing posture t work.	2–4
3	Moderate demand g handling of light t moderately heavy articles standing working or comparabl working posture	5–8
4	High demand *e g* handling f heavy to very heavy articles or strenuous work in an inconvenient posture	9–13
5	Higher demands, *e.g* handling of very heavy articles or trenuous work in an u*comfortable* posture	14–18

CO-OPERATION COMMITTEE

In addition to the work evaluation committee there is a co-operation committee consisting of three members from the management of the company and three from the trade unions. In this committee fundamental questions are discussed and agreed upon concerning the evaluation of the work and eval a tions which either party may consider erroneous.

EVALUATION OF MERITS

GENERAL

The evaluation of merits is an aid in th evaluation f work in the determina tion of wages. In the evaluation of work only the requirements placed by stand ard performance on the worker (skill, responsibility etc.) are considered. The evaluation of merits, on the other hand, is method for ystematic analysis and evaluation of the performance f the individual workers with respect to the fact rs incl ded in the merit evaluation system. It is thus not th person or the personal properties or the power f performance that are evaluated but only the ctual perf rmance

The evaluation of merits thus purs the w rkers to do their best.

FACTORS

ASEA merit valuation sy tem in ludes the following fact rs

1 Quantity
 Quality
3 Equipment and material,
1 Presence
 Knowledge
6. Duration of emplo ment.

Quantity i an expression for the rapidit with whi h the worker can do his work. For w rk that ha been evaluated on the basi f time-motion tudies, quantity is measured as the output per un t f time For employees on piece work the amount of work performed should be about 20 % higher than that for employees on time-work.

Quality i.e quality of work performed. Quality is judged independently of the risk of rejects, but in the calculation the risk f rejects is considered i.e whether it is gross, average or small. Only rejects for which the worker himself is responsible reduce the worker s score. Ex aggerated accuracy particularly if it is attained at the expence of quantity does n t increase the worker s score

In the facto *equ pment and material* the w rker is given points f the care with which he uses the tools machines and other equipment and how careful he is with th material. As in the f ctor Q ality this factor is judged independ ently f the risks.

The factor *Presence* evalu tes the time w rked with rdinary w rking time To facilitat evaluation, this facto is graded according t the number of days of absence Absence lowering the points for this fact r are absence with permission, oluntary absenteeism nd waiting days in the event of disease Absence because of attendence at committees f trade unions does not reduce the score for this fact r

Knowledge is evalu ted according to the potential value f the workers fo the company judged by his technical and occupational knowledge and possibilities f ad ancement.

INFORMATION

Before work evaluation is introduced *in a company and also before* evaluation of the work is started all those who might later have anything to do with the evaluation of the work should be informed of the principles according to which the work is evaluated and of the system used This applies particularly to *representatives of the trade unions* foremen, time-motion engineers and the like

KEY PROFESSIONS

In the evaluation of different types of work the examiners should first confine themselves to a certain number of key-occupations These key-occupations *should form the basis for comparison in* the evaluation of other occupations They should be representative of the company in question and they should as far as possible, cover all degrees of the factors included in the evaluation and be fairly evenly distributed within each evaluation factor

As to the number of key-occupations, this varies of course with the size of the company in which the evaluation of the work is to be applied At ASEA thirty *occupations were selected.*

LIST OF KEY OCCUPATIONS IN ASEA

Machine moulder operator
Hydro Blast operator
Knock out machine operator
Punch press operator
Chrome plater
Line assembler
Stock room tender
Truck driver
Janitor
Guard
Electric welder
Circular grinding machine operator
Spray painter
Turrect lathe operator
Coil winder (rotor)
Inspector
Steel treater
Floor moulder
Tool maker
Maintenance man
Rigger
Thick sheet metal worker
Thin sheet metal worker
Lay out man
Wood pattern maker
Machine assembler
Field assembler
Electrician
Electric tester

EVALUATION COMMITTEE

In order to obtain a comprehensive view of the work in the evaluation the latter was done by a committee.

The work evaluation committe at ASEA consists of 8 members. Five of them are permanent members i.e. they take part in the evaluation of all jobs The other three take part only in the evaluation of the work for the workshop in which they themselves are employed

WORK OF COMMITTEE

All the members in the committee receive a copy of the work to be evaluated The work of a given occupation i evaluated in the following way

A description of the work is carefully read, and in the event of doubt on any point, the work is studied at the workshop in question The work is evaluated by establishing which degree of the different factors agrees best with requirements of the work, after which the *number of points* for the factor i established The evaluati n of the key industries in the different factors i then used for comparison *The evaluation* committee then meets *for* di cu ion and establishment of the number of points

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FACTOR GRADES

Each factor is divided into five grades with an extra grade between each grade which in reality means a nine grade scale

The definition of the grades can be described in different ways according to the working place A suitable description for factors 1—5 is as follows

Grade 1 Unsatisfactory
Grade 2 Below normal,
Grade 3 Normal,
Grade 4 Above normal
Grade 5 Excellent

If measurable and reliable data are available for evaluation they should be used

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Page 19, left column, line 15 week *should read* year
25, left column, line 5 from below *g. should read g 3*
40, left column, line 4 9 *should read* 11
49 right column, line 4 from below VIII *should read* VII
64, left column, line 9 from below 9/12² *should read* (9/12)
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66, Table 25, significance 1.01 *should read* 0.01
69, Table 26, column 2 59 57 etc *should read* 1959 1957 etc.
71, Fig 24 The formula *should read* exactly as on page 70 right column line 20 from below
73, right column, line 8 from below data machine *should read* computer
76, left column, line 1 strips *should read* tapes
76, left column, line 2 and 3 stamped *should read* punched
76, right column, line 3 *plus should read* marked plus sign
86, left column, line 12 from below 1951 *should read* 1961
103, right column *Delete* line 7 from below
104, Table 36, line 2 from below 1.9 m *should read* 1.9 m²
105, left column, line 1 LINDEGARD *should read* LINDEGARD MORLING & NYMAN
106, left column, line 1 from below haemoglobulin *should read* haemoglobin
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125, left column, line 6 from below 121 *should read* 123
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129 left column, line 16 from below 125 *should read* 123
130, right column *Delete* line 9 from below
134, line 1 VIII *should read* XI
134, line 5 VI *should read* XI
135, left column, line the muscle factor and fat factor *should read* some of the muscle factor and fat factor variables
137, right column, line from below 14% The *should read* 11% and the
137 right column, line 5 from below 2.2, 2.2 and 6.6 *should read* 3.0, 1. and 4.2
138, left column, line 1 Lindegard *should read* LINDEGARD
143, right column, line 7 T-test *should read* -test
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156, left column, line 5 and 13 data machine *should read* computer
158, left column, line 1 *Delete* the industries
169 *Add* ALLARDT E Drinking norms and drinking habits. The Finnish Foundation for Alcohol Studies publ no 6 1957
170, *Add* FOR MAN Industrial Health in Industrial and Nonindustrial Countries. A Comparative Study Journal of Occupational Medicine Vol 1 no 1 1959
170, *Add* DE GROOTH *See page* 171 Nederlands tijdschrift etc.
171, left column, line 22 52.10 *should read* 5..9
171, *Add* LINDEGARD B, MORLING G, NYMAN E Male Sex Characters in Relation to Bodybuild, Endocrine Activity and Personality Lund universitets årsskrift N.F ... 5..10.1956
171, *Add* LINDEGARD B En antropologisk undersökning av den normala parkaktiviteten hos svenska män ... Nord. psykiatr medlemsblad, 1.. 19..
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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 376

IRON ABSORPTION STUDIES II

H. BRISE AND L. HALLBERG: A method for comparative studies on iron absorption in man using two radioiron isotopes.

H. BRISE AND L. HALLBERG: Absorbability of different iron compounds.

H. BRISE: Influence of meals on iron absorption in oral iron therapy.

H. BRISE: Effect of surface-active agents on iron absorption.

H. BRISE AND L. HALLBERG: Effect of ascorbic acid on iron absorption.

H. BRISE AND L. HALLBERG: Effect of succinic acid on iron absorption.



FROM THE DEPARTMENT OF MEDICINE II (HEAD: PROFESSOR ERIK W ...), UNIVERSITY OF GÖTEBORG,
SAHLGRENSKA SJUKHUSET GÖTEBORG, SWEDEN.

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FROM THE ARTMENT OF MEDICIN II (HEAD: PROFESSOR ERIK WARÉ), UNIVERSITY OF GOTEBORG,
AHLGRENS SJUKH ET GÖTEBORG, SWEDEN

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II*

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A series of papers has arlier been published under the heading Iron Absorp ion Studies Supplement 358 t olume 168, 1960 of

ACTA MEDICA SCANDINAVICA

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A METHOD FOR COMPARATIVE STUDIES ON IRON ABSORPTION IN MAN USING TWO RADIOIRON ISOTOPES

By

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INTRODUCTION

The amount of iron absorbed at one occasion can be accurately determined using available radioiron methods[1, 2, 3]. In comparative studies (e.g. the absorbability of different iron compounds) other methodological problems will arise. When comparing the absorption of iron in two groups of individuals treated in different ways the great variation in absorption between individuals will make the results from such studies very difficult to interpret even when using accurate methods to determine the absorption. Because of this, comparisons have often been made in the same subject. However, great variation in the absorption of iron occurs not only between individuals but also within a single individual on different days. Both these sources of variation were considered when the present method for comparative studies on iron absorption was designed.

The method was based on a repeated administration of iron to each individual (one dose on each of 10 days), giving on alternate days two iron compounds each compound labelled with a different radioiron isotope (Fe^{55} or Fe^{59}). Determinations of Fe^{55} and Fe^{59} activities were made in a blood sample drawn two weeks after the last oral iron dose when an optimal utilization of absorbed iron for hemoglobin synthesis could be expected to have taken place[4–]. From these determinations the relative absorbability of the two compounds could be calculated. By giving repeated iron doses (5 doses of each compound on alternate days) the error due to the variation in absorption on different days could be reduced by more than half, and by making the comparison within the same subject the variation in absorption between individuals was eliminated.

In 1958 a preliminary report was given on the method. In the present paper the details of the experimental procedure are given and the validity of the method is more thoroughly tested. As an example of the application of the method a study of the relative absorbability of ferrous and ferric iron is included.

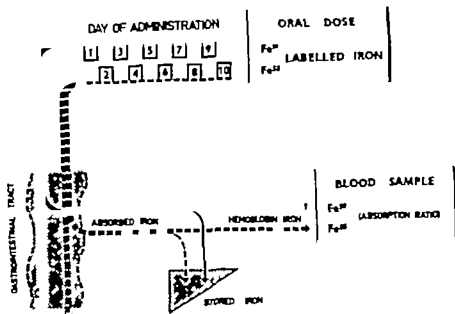


Fig 1 Experiment 1 design.

Freshly boiled distilled water w used in the preparation of the solution and nitrogen a bubbled through the solutions in the fla ks before closing. The ferric iron content in the ferrous sulphate solution in these 25 ml flasks wa les than 1 per cent fter 4 6 week storage in room t mperat re

The Fe^{55} and Fe^{59} wa btained from Abbott Laboratories Oak Ridge Tennessee U S A solution of $FeCl_3$ (pH less than 1 5) The pecific activity of Fe^{59} w 5 13 m rocuries per microgram and the pecific act vit of Fe^{55} a —3 microcuries per microgram respectively Stock solution f Fe^{55} nd Fe^{59} in 0.02 N HCl containing 3 μC of radioiron per ml ere prepared from the original solution

The final pH in the administered solu tions w —4. The total mou t of rad activity admini tered to each subject was less than —5 μC Fe^{59} and —5 μC Fe^{55}

As ferric chloride wa used to label the ferrous sulphate the isotope exchange was tested. An cid solution containing the two compound wa transferred to a separatory funnel nd extracted with isopropylether which will extract only ferric ions under the condition used A complet xchange wa found t have taken place the radioactivity in the isopropylether layer wa less than per cent of the original amount

RADIOACTIVE ANALYSIS

From the blood sample (150 ml) drawn in 50 ml ACD-solution) week after the last oral iron dose four samples each

) 1 32 g of sodium citrat g of citric acid 1 g of glucose t 100 ml with water

MATERIAL AND METHODS

MATERIAL

Sixtytwo subjects were included in this study One subject (I M T) had a hypernephroma without demonstrable metastasis One subject (20-M BII) had a Bilroth II gastric resection several years ago *Three subjects had an iron deficiency anemia after acute blood loss (ID)* The other subjects were healthy volunteers (N) some of whom had served as blood donors (BD) In the tables (M) denotes male and (F) female subjects.

PRINCIPLE OF METHOD AND EXPERIMENTAL PROCEDURE

The experimental design is outlined in figure 1 Unless otherwise stated the same amount of elemental iron was given every morning for 10 days after an overnight fast The iron was labelled with Fe^{55} and Fe^{59} on alternate days When comparing ferric and ferrous iron for instance the compounds were labelled with different radioiron isotopes and were given on alternate days. *To reduce systematic errors the first iron dose was alternately labelled with* Fe^{55} *and* Fe^{59} and was also alternately ferrous and ferric iron From analysis of Fe^{55} and Fe^{59} activity in a blood sample drawn 2 weeks after the last oral dose the mean absorption ratio was calculated

Each subject received a box containing 10 consecutively numbered 25 ml flasks which were taken in order Detailed written and oral instructions were given for the experiment

The iron solution was taken directly from the flask This was then filled with tap water and the rinse water was also taken This procedure was repeated so that the *total volume consumed* was 75 ml No food or drink was taken for an additional two hours.

The residual radioactivity in the flasks was less than 0 5 per cent of the original content This determination was made using a scintillation detector with a 3 inch × 6 inch. plastic crystal with a well to contain the whole flask

ORAL IRON DOSES

The ferrous iron in this study was $FeSO_4 \cdot 7H_2O$ (Merck pro analysi) The ferric iron content was less than 1 per cent as found by analysis using the thiocyanate method[19]

The ferric iron salt administered was $Fe_2(SO_4)_3 \cdot 6H_2O$ (Union Chimique Belge pour analyse).

The volume of the solution in each flask was 25 ml and contained 30 mg of elemental iron 10 mg of ascorbic acid (to prevent oxidation of ferrous iron — no ascorbic acid was added to ferric iron solutions) 4 gram of sucrose and 4—7 μC of radioiron In the solution containing 5 mg of iron the ascorbic acid content was reduced proportionally to 1.23 mg

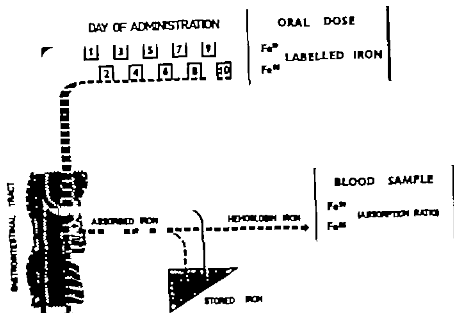


Fig. 1 Experimental design.

Freshly boiled distilled water was used in the preparation of the solutions and nitrogen was bubbled through the solutions in the flasks before closing The ferric iron content in the ferrous sulphat solutions in these 25 ml flasks was less than 1 per cent after 4—6 weeks storage in room temperature

Th Fe^{55} and Fe^{59} was obtained from Abbott Laboratories, Oak Ridge Tennessee U.S.A. as a solution of $FeCl_3$ (pH less than 1.5) The pecific activity of Fe^{55} was 3—13 microcuries per microgram and the specific activity of Fe^{59} was 2—3 microcuries per microgram respectively Stock solutions of Fe^{55} and Fe^{59} in 0 01 N HCl containing —3 µC of radioiron per ml were prepared from the original solutions.

The final pH in the administered solutions was —4. The total amount of radioactivity administered t each subject was less than .5 µC Fe^{5} and 25 µC Fe^{55}

As ferric chloride was used to label the ferrous sulphate the isotope exchange was tested. An acid solution containing the two compounds was transferred to a separatory funnel and extracted with isopropylether which will extract only ferric ions under the conditions used A complet exchange was found to have taken place as the radioactivity in the isopropylether layer was less than per cent of th original mount.

RADIOACTIVE ANALYSIS

From the blood sample (150 ml) drawn in 50 ml ACD-solution) * weeks after th last oral iron dose four samples each

) 1.32 g of sodium citrate, 0 8 g of citric acid, 1. 7 g of glucose to 100 ml with ater

containing *5 mg of iron* were *digested*, the iron electroplated and the Fe^{55} and Fe^{59} activity determined as previously described[11] The mean values obtained were used.

Standard solutions were prepared from the described stock radioiron solutions Two milliliters of stock solution were transferred 4 times to a 1 000 ml measuring flask using the same pipette as that used in preparing the oral doses The flasks were then filled up with 0 02 N HCl.

A known amount of the standard solution was digested with inert iron (to give *5 mg of iron*) and *electroplated together* with the unknown samples From each standard solution 6 electroplated reference samples were made The unknown samples were measured together with the reference samples in an automatic sample changer

CALCULATIONS

The Fe^{55} and Fe^{59} activities per 5 mg of iron in circulating red *cells* were determined according to formulas given in an earlier paper[11]

The amount of absorbed iron *labelled* with Fe^{55} or with Fe^{59} in circulating red cells was calculated from the activities of Fe^{55} and Fe^{59} in the administered doses and from the activities in the blood according to the following equation.

$$\frac{a \cdot D}{100} = F \cdot TFe \qquad 1$$

where

a — per cent of the administered iron in *the circulating hemoglobin* mass

D — administered radioactivity (Fe^{55} or Fe^{59})

F — observed radioactivity (Fe^{55} or Fe^{59}) per milligram of iron in blood

TFe = total amount of circulating hemoglobin iron in milligrams

TFe was calculated from the estimated blood volume (males = weight in kilograms × 74 2· females = weight in kilograms × 65)[26] the hemoglobin concentration in the blood and with the presumption *that 1 g hemoglobin contains 3 34 milli*grams of iron accordingly Hemoglobin was determined as cyanmethemoglobin[27]

$$TFe = \frac{BV \cdot Hb \cdot 3.34}{100} \qquad 2$$

where

BV — estimated blood volume in milliliters

Hb — hemoglobin concentration in grams per 100 ml blood

When two compounds were compared in this experimental design a figure of the relative absorbability of the two compounds was *obtained according to the* following equations

$$A = \frac{a}{k} \qquad 3$$

where

A — *total amount of* absorbed iron in per cent of the amount administered

k = fraction of absorbed iron *in the* circulating hemoglobin mass

a was calculated from Equation 1 and

Absorption ratio

$$\frac{A_{55}}{A_{59}} = \frac{F_{55}\ D_{59}\ k_{59}}{F_{59}\ D_{55}\ k_{55}} \qquad 4$$

where

A_{55} and A_{59} = total amount of absorbed iron labelled with Fe^{55} and Fe^{59} respectively in per cent of the amount of iron administered,

and,

D_{55} and D_{59} = total amount of administered Fe^{55} and Fe^{59} respectively

Assuming that the difference of the average internal distribution of absorbed iron on different days is negligible (i.e. $k_{59} = k_{55}$) the absorption ratio can be calculated from the simplified equation.

$$\text{Absorption ratio} = \frac{A_{55}}{A_{59}} = \frac{F_{55}\ D_{59}}{F_{59}\ D_{55}} \qquad 5$$

It is obvious that the accuracy of the estimation of TFe does not influence the accuracy of the absorption ratio. The figures for Absorption given in the tables are calculated from the estimates of TFe Because of this fact these figures are not true expressions for the total absorption since only the absorbed iron utilized for red cell formation is included. The Absorption figures are given only to facilitate comparisons between individuals.

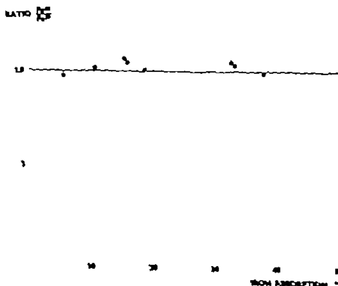


Fig. 2. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphat administered on alternate days for 10 days. (Each dose was equivalent to 30 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots in those starting with Fe^{59} as rings

containing 5 mg of iron were digested the iron electroplated and the Fe^{55} and Fe^{59} activity determined as previously described[11] The mean values obtained were used

Standard solutions were prepared from the described stock radioiron solutions. Two milliliters of stock solution were transferred 4 times to a 1 000 ml measuring flask using the same pipette as that used in preparing the oral doses The flasks were then filled up with 0 02 N HCl.

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The amount of absorbed iron labelled with Fe^{55} or with Fe^{59} in circulating red cells was calculated from the activities of Fe^{55} and Fe^{59} in the administered doses, and from the activities in the blood according to the following equation.

$$\frac{a \cdot D}{100} = F \cdot TFe \qquad 1$$

where

a = per cent of the administered iron in the circulating hemoglobin mass

D = administered radioactivity (Fe^{55} or Fe^{59})

F = observed radioactivity (Fe^{55} or Fe^{59}) per milligram of iron in blood

TFe = total amount of circulating hemoglobin iron in milligrams

TFe was calculated from the estimated blood volume (males = weight in kilograms × 74 2, females = weight in kilograms × 65)[14] the hemoglobin concentration in the blood and with the presumption that 1 g hemoglobin contains 3 34 milligrams of iron accordingly Hemoglobin was determined as cyanmethemoglobin[22]

$$TFe = \frac{BV \cdot Hb \cdot 3\,34}{100} \qquad 2$$

where

BV = estimated blood volume in milliliters

Hb = hemoglobin concentration in grams per 100 ml blood

When two compounds were compared in this experimental design a figure of the relative absorbability of the two compounds was obtained according to the following equations

$$A = \frac{a}{k} \qquad 3$$

where

A = total amount of absorbed iron in per cent of the amount administered

k = fraction of absorbed iron in the circulating hemoglobin mass

a was calculated from Equation 1 and,

RATIO $\frac{Fe^{55}}{Fe^{59}}$

1.0

0.5

10 20 30 40 50

IRON ABSORPTION %

Fig. 3 Analysis of experimental error of method. Absorption ratio of Fe^{55} and Fe^{59} labelled iron from single dose (30 mg F) containing known amount of both isotopes Result plotted against estimated absorption.

Ferrous sulphat was given as a solution containing 30 mg of elemental iron on each of 10 days labelled with Fe^{55} or Fe^{59} respectively on alternate days. Ten subjects st rted with Fe^{55} labelled iron and fourteen subjects with Fe^{59} labelled iron.

The btained result are given in table I and graphed in figure 2 The mean value of the bsorption ratio (Fe^{55}/Fe^{59} labelled iron) in those subjects starting with Fe^{55} labelled iron wa the same as in those starting with Fe^{59} labelled iron (1 00 and 1 01 respectively)

T be able to calculate that part of th variation which is due to a varying absorption nd internal distribution of iron on d fferent days the experimental error was calculated in the following way

Nine subjects were given one 30 mg iron dose of ferrous sulphate labelled with known amounts of both Fe^{55} and Fe^{59} A blood sample was drawn weeks later and the bsorption ratio was calculated as described previously The results are given in table II and in figure 3

The standard error was ± 2.2 per cent Because the ratio Fe^{55}/Fe^{59} must be the sam in the blood sample as in the dose administered in this experimental design the calculated standard error must be identical with the experimental error of the method.

This experimental error does not include the variation of emptying nd rinsing of the flasks containing the iron doses. However this latter error is quit neglectible

RESULTS

CONTROL STUDIES

Even when the foregoing experimental design is used, the accuracy of comparisons of the absorbability of different iron compounds is limited by (a) the day to day variation in the absorption of iron and (b) the variation of the internal distribution of the absorbed iron to erythropoiesis and storage In order to be able to calculate the magnitude of the total variation the following studies were made

TABLE I

Precision and accuracy of method Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days Each dose equivalent to 30 mg of elemental iron

SUBJECT	First dose	"ABSORPTION" (per cent) Fe^{55}	Fe^{59}	ABSORPTION RATIO Fe^{55}/Fe^{59} Individual value	Mean value
1 M T	Fe^{55}	0 7	0 9	0 [illegible]	
2 M N	Fe^{55}	8.7	7 8	1 11	
3 M BD	Fe^{55}	8.5	8 7	0 98	
4-M N	Fe^{55}	1 [illegible]	11 4	1 06	
5-M BD	Fe^{55}	13.[illegible]	1 [illegible]	1 04	
6 M BD	Fe^{55}	18 9	18.8	1 01	
7 M BD	Fe^{55}	24 2	22 7	1 03	
8-M BD	Fe^{55}	34 2	33 3	1 03	
9-F BD	Fe^{55}	30 8	34 8	0 [illegible]	
10-M BD	Fe^{55}	3 [illegible]	3 9	0.99	1 [illegible]
11 M N	Fe^{59}	3 1	3 2	0 97	
1[illegible] M N	Fe^{59}	5 [illegible]	3 7	0.9[illegible]	
13 M N	Fe^{59}	6 5	7	0 [illegible]	
14 F N	Fe^{59}	11 3	9 8	1 1[illegible]	
15-M BD	Fe[illegible]	11 1	10 8	1 [illegible]	
16-M BD	Fe^{59}	15 4	15 0	1 03	
17 F BD	Fe^{59}	16 7	15 6	1 07	
18 F BD	Fe^{59}	16 8	16 1	1 05	
19 M BD	Fe^{59}	18 1	1 [illegible]	1 06	
[illegible]0 M BD	Fe^{59}	5 1	4 4	1 07	
[illegible]1 F N	Fe^{59}	23 9	23.6	0 95	
[illegible] M BD	Fe^{59}	20 2	3 [illegible]	0.80	
23 M BD	Fe^{59}	29 0	27 9	1 [illegible]	
[illegible]4 M BD	Fe^{59}	46.1	41 2	1 12	1 [illegible]

Absorption ratio Mean value: 1 [illegible]
Standard error of mean ±0 [illegible]
Standard error ±0.[illegible]

RATIO $\frac{Fe^{59}}{Fe^{55}}$

1.5

1

20 40 60 80

IRON ABSORPTION %

Fig. 4. Precision and accuracy of method. Absorption ratio of Fe^{59} labelled and Fe^{55} labelled ferrous sulphate administered on alternat days for 10 days. (Each dose was equivalent to 5 mg of elemental iron.) Observed bsorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{59} were indicated as dots in those starting with Fe^{55} as rings

As S is obtained from a mean value f 5 pairs of comparisons the variation in absorption and utilization of absorbed iron from one day to another within th single individual can be calculated as $\sqrt{5 \times 0.007880} = \pm 20$ per cent (coefficient of variation).

The variation in bsorption on different days was lso studied when using 5 mg doses, beca se such a dose is more closely related to physiological conditions and has been recommended as the most satisfactory dose for testing iron absorption[23].

In this series comprising 10 subjects the 5 mg iron dose was given for 10 days in the same way as in the first series. The results obtained are given in table III and figure 4.

The observed standard deviation of the absorption ratio was 16 per cent. By resolution of the variance in the two components as bove the variation in bsorption on different days within the single individual using 5 mg doses was $\sqrt{5 \times 0.012784} = \pm 25$ per cent (coefficient of variation). As found by an F-test the standard deviation was greater when 5 mg doses were used than when 30 mg doses were used ($p < 0.05$).

as found by analysis of remaining radioactivity in the flasks (less than 0 5 per cent)

From the figures obtained for the total variation S_t (Variance = 0 00801 S = ± 0 09 — see table I) and experimental error S_{exp} (Variance = 0 000487 S = ± 0 0 — see table II) it is possible to calculate the sum of the real variation in absorption and internal distribution of absorbed iron (S) using the following formula for resolution of a variance in two components

$$S_t^2 = S_{exp}^2 + S^2$$

The calculated real variation in the absorption and distribution of absorbed iron was thus found to be ±0 per cent — variance 0 007530 This means that the experimental error only forms a negligible part of the total variation

TABLE II

Analysis of experimental error of method Administration of a single iron dose (30 mg Fe) containing known amounts of Fe^{55} and Fe^{59}

SUBJECT	ABSORPTION" (per cent) Fe^{55}	Fe^{59}	ABSORPTION RATIO Fe^{55}/Fe^{59}
-5-M N	0.	0 7	0 94
6-M B H	1 8	1 9	0.98
27 M N	-1	1	1 0
8 M N	7 2	7 1	1 0
"9 F N	8	8 1	1 02
30-F N	11 3	11 1	1 0
31 F ID	5.2	-3 9	1 01
3 F ID	2-3	2-5	0.98
33-M ID	53.0	53 5	0.99

Absorption ratio: Mean value: 1 00
Standard error of mean: ±0.01
Standard error: ±0 0

TABLE III

Precision and accuracy of method Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days Each dose equivalent to 6 mg of elemental iron

SUBJECT	First dose	"ABSORPTION" (per cent) Fe^{55}	Fe^{59}	ABSORPTION RATIO Fe^{55}/Fe^{59} Individual value	Mean value
34-M N	Fe^{55}	7 6	6	1 01	
35-M N	Fe^{55}	9 1	7 8	1 17	
36-M N	Fe^{55}	9 9	9 0	1 10	
37 M BD	Fe^{55}	19 9	17 8	1 12	
38-M BD	Fe^{59}	2 5	23 7	1 05	1 09
39 M N	Fe^{59}	12 0	10 5	1 3	
40-F N	Fe^{59}	10 8	1 3	1 14	
41 M BD	Fe^{59}	4" 2	2-1	0 5	
4 F BD	Fe^{59}	4 0	61 6	0. 6	
43-M BD	Fe^{59}	66 0	78.7	0 4	0 94

Absorption ratio Mean value 1 03
Standard error of mean: ±0.0
Standard error ±0 16

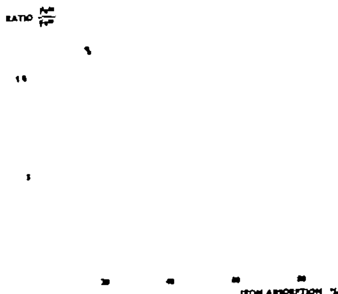


Fig. 4. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. (Each dose was equivalent to 5 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots, in those starting with Fe^{59} as rings ∘.

As S_x is obtained from a mean value of 5 pairs of comparisons the variation in absorption and utilization of absorbed iron from one day to another within the single individual can be calculated as $\sqrt{5 \times 0.007830} = \pm 20$ per cent (coefficient of variation).

The variation in absorption on different days was also studied when using 5 mg doses, because such a dose is more closely related to physiological conditions and has been recommended as the most satisfactory dose for testing iron absorption[12].

In this series comprising 10 subjects the 5 mg iron dose was given for 10 days in the same way as in the first series. The results obtained are given in table III and figure 4.

The observed standard deviation of the absorption ratio was 16 per cent. By resolution of the variance in the two components as above the variation in absorption on different days within the single individual using 5 mg doses was $\sqrt{5 \times 0.024794} = \pm 35$ per cent (coefficient of variation). As found by an F-test the standard deviation was greater when 5 mg doses were used than when 30 mg doses were used ($p < 0.05$).

RATIO $\frac{Fe^{+++}}{Fe^{++}}$

1.0

0.5

10 20 30 40 50

IRON ABSORPTION %

Fig 5 Absorption of ferric versus ferrous iron at different estimated absorption levels Each iron dose was equivalent to 40 mg of elemental iron. (● indicates subject starting with ferrous sulphate, ○ indicates subject starting with ferric sulphate)

COMPARISON OF THE ABSORPTION OF IRON FROM FERROUS AND FERRIC SULPHATE

As an example of the application of this double isotope method a comparison of the absorption of iron from ferrous and ferric sulphate is included in the present paper

It has repeatedly been shown, using different methods that ferrous iron is more readily absorbed than ferric iron[14—17] Because of this a comparison of ferrous and ferric iron may also serve as an indirect check of the method The data on the quantitative importance of the valency of iron are greatly diverging The present method can be expected to give more exact information on the long debated problem

a. *Oral iron dose 30 mg*

Eight subjects were included in this study The solutions were prepared as previously described (ascorbic acid was not added to the ferric sulphate solutions) and each dose contained 30 mg of elemental iron In five subjects the ferrous iron was labelled with Fe^{59} in three subjects with Fe^{55} In order to further reduce the possibility of systematic errors in this comparison 5 subjects started with the ferrous dose and 3 subjects with the ferric dose

The results are given in table IV and are illustrated in figure 5 In the figure the absorption ratio ferric ferrous iron is graphed against the absorption of iron from the ferrous sulphate solution The term absorption is used to mean the

TABLE IV

Absorbability of ferric and ferrous sulphate. (Each dose equivalent to 30 mg of elemental iron.)

SUBJECT	First dose	ABSORPTION (per cent)		ABSORPTION RATIO Ferric/Ferrous iron
		Ferric iron	Ferrous iron	
44-M X	Ferrous iron	0	2.2	0.28
45-M X	Ferric iron	1.1	3.4	0.32
46-M BD	Ferric iron	2.6	7.2	0.4
47-M BD	Ferrous iron	1	7	0.2
48-F BD	Ferrous iron	4	8.	0 [illegible]
49-M BD	Ferric iron	5.2	18.0	0.34
50-M BD	Ferrous iron	6.9	24.0	0.25
51-M BD	Ferrous iron	8.7	23.7	0.[illegible]

Absorption ratio: Mean value: 0.34
Standard error of mean: ±0.03
Standard error: ±0.09

PERCENT

100 –

FeSO4

50

30 90 150 210

IRON DOSE mg

Fig. 6. Absorption of ferrous and ferric iron at different dosage levels. The same amount of iron was given each day. Each line represents absorption of ferric iron as percentage of the absorption of ferrous iron in the same subject.

per cent of absorbed iron in circulating red cells 2 weeks after the administration of the last oral iron dose

The mean value of the absorption ratios in these 8 subjects was 0 34 ± 0 03 and it is thus quite clear that ferrous iron is much more readily absorbed than ferric iron.

b Oral iron dose 90—210 mg

It is possible that the magnitude of the iron dose may influence the relative absorbability of ferrous and ferric iron. An additional study was thus made in which ferrous and ferric iron were compared at higher dose levels (90 150 and 210 mg of elemental iron) The same amounts of ferrous and ferric iron were thus given to each subject

The results are given in table V and are also graphed in figure 6 where each bar represents one subject It is evident that the greater absorbability of ferrous iron was more pronounced the higher the iron dose A correlation analysis between absorption ratio and iron dose gave the following results $r = -0\ 50$ and $p < 0\ 05$

When 30 mg iron doses were compared 3 times more ferrous iron was absorbed When 90 150 and 210 mg doses of iron were studied respectively 4 5 and times more ferrous than ferric iron was absorbed

TABLE V

Absorbability of ferric and ferrous sulphate at different dose levels (90 — 10 mg)

Daily oral dose (mg F)	SUBJECT	First dose	ABSORPTION (per cent)		ABSORPTION RATIO Ferric Ferrous iron	
			Ferric iron	Ferrous iron	Individual value	Mean value
00	3 M BD	Ferric	1 0	3.0	0 33	
00	53-M BD	Ferric	1 4	3	0 2	
90	34 M BD	Ferrous		1	0 21	
00	35-F BD	Ferrou	3 1	16 4	0 2	0 2
130	56-M BD	Ferrous	1 3	6 1	0 21	
130	7 M BD	Ferric	1	7	0 13	
130	38-M BD	Ferric	1	8 3	0 20	
150	39 M BD	Ferrous	3	23 1	0 3	0 21
10	60-M BD	Ferrous	0.3	4 4	0 10	
10	61 M BD	Ferrous	1 0	6.1	0 17	
10	62 M BD	Ferric	1 2	8	0 14	0 14

COMMENT

This method was devised in an attempt (a) to make more valid comparisons of the absorption of iron from different iron compounds and (b) to facilitate the quantitation of factors influencing the absorption of iron. An example of th latter application of the method is study of the effect of meals on iron absorption presented in a preliminary report[3]

Earlier comparative studies have almost exclusively been devoted to the relative bsorbability of different iron compounds [4-8] The comparisons have usually been based on determinations of the regeneration rate of hemoglobin during iron therapy in iron deficient subjects. Two or more groups of subjects treated with different iron compounds have been compared However there re numerous factors influencing the therapeutic response t iron (severity of anemia, continued bleed ing condition of iron stores infections etc.) which often make such comparisons difficult to interpret and necessitate comparisons between large homogenous materials

Using the method described in this paper the main sources of error in comparative studies on iron absorption are greatly diminished The repeated administration of iron reduces the average variation in bsorption and internal distribution of bsorbed iron within the single individual t less than one half, since the absorption ratio is a mean value of five pairs of comparisons. Inasmuch a the ingle subject serves as his own control valid conclusions can be drawn from materials containing relatively few individ ual For the same reason the requirements of a selection and classification of subject for comparative iron absorption studies are also markedly reduced.

The method is convenient ince it is not necessary to quantitate the total absorption (e.g by faeces collection) to be able to study the effect of a substance on iron absorption or the relative absorbability of iron from two compounds

Iron doses labelled with different isotopes were not given on the same day in order to diminish the possibility of an exchange of radioiron between different doses in that part of the intestine where a measurable absorption could take place

The effect of a preceding dose on the absorption of a subsequent dose was found to be negligible in this experimental design since the mean value of the obtained absorption ratio (Fe^{55} labelled ferrous sulphate/Fe^{59} labelled ferrous sulphate) in the group starting with one isotope did not differ from the mean value in the group starting with the other isotope.

The 30 mg iron dose was most thoroughly studied because it can be considered to be a therapeutic oral dose. The 5 mg iron dose was also studied inasmuch as it may represent an optimal physiological iron dose. The observed greater variation in the absorption of this small dose may be explained by the relatively greater influence of extraneous random factors (g adsorption to mucin or protein components in the gastrointestinal tract).

The sources of error in this method are of two kinds. One kind consist in analytical errors and errors in the administration of the iron doses. The magnitude of these

errors was found to be only about 2 5 per cent The other and main source of error is the variation in absorption and distribution of absorbed iron. This error can be further reduced only by giving more iron doses for longer time

In 10 subjects a blood sample was drawn not only 2 weeks after the last oral dose but also after 3 weeks and in 5 of the subjects at times up to 2 months after the last dose. The difference between the absorption ratios within the single subject was of the same magnitude as the experimental error The effect of a variation in internal distribution of absorbed iron on the real absorption ratio can be expected to decrease in time The fact that no significant difference between absorption ratios was found, when followed for longer time indicates that the main part of the observed total variation is related to a variation in absorption from day to day This variation was about ± 20 per cent when 30 mg iron doses were given and about ± 35 per cent when 5 mg doses were given This great variation means that it is very difficult or impossible to demonstrate minor differences in absorbability of two compounds if such a comparison is based on determinations of iron absorption on two occasions in the same subject even if these determinations are made with a very accurate method The great variation in absorption of iron on different days in the same subject stresses the importance of giving iron in repeated doses in comparative studies in the same individual

The degree of underestimation of the real absorption as calculated from the radioactivity in the red cell mass 2 weeks after the last oral dose does not influence the absorption ratio These "absorption figures" have only been given as a rough classification of the subjects avidity to absorb iron

The observed lower absorption of ferric iron (compared with ferrous) is consistent with earlier observations[11–17] From the observed difference in absorbability it is not necessary to postulate that iron is absorbed only in the ferrous state The difference can most easily be explained from the well known physico-chemical difference between ferric and ferrous ions. At the pH existing in the gastrointestinal tract, a considerably greater amount of the ferric than of the ferrous iron will be present as undissociated hydroxide Moreover ferric iron has a greater avidity to form insoluble compounds or complex compounds than ferrous iron The average ionic concentration of iron in the upper part of the intestinal tract where the absorption of iron mainly takes place can thus be expected to be much higher when ferrous iron is given than when ferric iron is given

The difference in relative absorbability between ferrous and ferric iron can be expected to be more pronounced the higher the iron dose because at higher dose levels the ferric ion concentration will remain constant while more and more undissociated ferric hydroxide will be formed

This reasoning is consistent with the observed decrease of the ferric ferrous iron absorption ratio with increased iron doses (0 34—0 14 at the dose levels 30 and ?10 mg of iron respectively)

It is also consistent with the observation by BONNET HAGEDORN and OWEN who found no difference in absorbability of ferrous and ferric iron when very small amounts (50 μg) of elemental iron were

used[*]. At the much lower concentration of iron achieved in the gastrointestinal tract with this extremely small iron dose, it can be expected that ferrous and ferric iron will both be present in ionic form to the same degree (the solubility product of ferric hydroxide will not be exceeded).

From the present studies it can be concluded that considerably more iron is absorbed from ferrous than from ferric sulphate — At therapeutic dose levels (30 mg of iron or more) at least 3 times more iron will be absorbed if given in the ferrous state. This difference in absorbability between ferrous and ferric iron is of such a magnitude that it can be concluded that ferric iron has no place in oral iron therapy.

SUMMARY

A method is described which is especially devised for comparative studies of the absorbability of different iron compounds and for quantitation of the influence of various factors on iron absorption.

Two radioiron isotopes are used — Fe^{55} and Fe^{59}. One iron compound is labelled with one isotope and one compound with the other. The compounds (and isotopes) are administered on alternate days for ten consecutive days.

From analysis of Fe^{55} and Fe^{59} in one blood sample drawn two weeks after the last oral dose the relative absorbability of different iron compounds can be determined.

By giving ferrous sulphate labelled with the two isotopes on alternate days the accuracy and precision of the method has been determined. The average day to day variation in absorption of iron in the single individual was found to be about ± 20 per cent using 30 mg doses and ± 35 per cent using 5 mg doses.

As an example of the application of the method the absorbability of iron from ferrous and ferric sulphate has been studied at different dosage levels. It was found that about 3—7 times more iron was absorbed from ferrous sulphate than from ferric sulphate.

The results show that the method will greatly facilitate comparative iron absorption studies since each subject serves as his own control.

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ABSORBABILITY OF DIFFERENT IRON COMPOUNDS

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

A great number of iron compounds are used in the treatment of iron deficiency. With the dosage commonly employed today side-effects are fairly uncommon. In a well controlled study of side-effects in oral iron therapy no significant difference was observed between some commonly used iron compounds when the same amount of elemental iron was given. With increasing dose of iron more side-effects are encountered[1]. Because of this, the therapeutic value of an iron compound is determined mainly by its absorbability. However, inasmuch as it is very difficult to compare the absorbability of iron compounds, it is also difficult to get an objective measurement of the therapeutic value of different iron compounds.

The absorbability of an iron compound has usually been evaluated from the regeneration rate of hemoglobin in cases with iron deficiency anemia during iron therapy[2–8]. However, the regeneration rate is not only determined by the amount of iron absorbed but also by a number of other factors such as the severity of anemia, the condition of the iron stores and the presence of concurrent other diseases (e.g. infections). A false value of the absorption can also be obtained if the amount of iron absorbed is greater than the amount utilized by the bone marrow. Such sources of error in estimating absorption of iron from the regeneration rate of hemoglobin will make it necessary to have fairly large homogenous materials when the absorbability of different iron compounds are to be compared.

Other methods which have been used to compare the absorbability of iron compounds (e.g. the plasma iron increase after oral administration of different compounds), also have considerable sources of error. These will be discussed later.

The difficulties in evaluating the relative absorbability of different iron compounds can perhaps account for the diverging results obtained by different authors and the numerous iron preparations used to-day, most of which have been introduced with claims of superiority. A critical review of the present confused situation has been published by BEUTLER 1960[9].

The double radioiron method offers the possibility of making a more valid evaluation of the absorbability of different iron compounds since two compounds can be compared in the same individual within the same period thereby reducing or eliminating some of the main sources of error in earlier methods[10,11,12] Actually the difficulties in comparing the absorbability of iron compounds initiated the devising of the double radioiron method.

In this paper the absorbability of 14 iron compounds are reported In each individual two compounds labelled with different radioiron isotopes have been compared — One of these two compounds has always been ferrous sulphate Thus ferrous sulphate served as a reference in all subjects. A preliminary report including only 4 compounds was published in 1958 as an example of possible applications of this double radio-isotope method[10,11]

METHODS AND MATERIAL

METHODS

The experimental design of this study and the details of the method were the same as previously described.[12] A solution of 25 ml containing 30 mg of elemental iron was given every morning after an overnight fast for 10 days Ferrous sulphate was labelled with one radioiron isotope and the iron compound under study was labelled with the other isotope The two compounds were given on alternate days This design (giving ferrous sulphate to all subjects on alternate days) will thus make possible a comparison between individuals by making ferrous sulphate a common reference

In order to reduce systematic errors the first dose was alternately ferrous sulphate and the compound under study and the ferrous sulphate was alternately labelled with Fe^{55} and Fe^{59} The solutions containing ferrous iron also contained 10 mg of ascorbic acid for every dose in order to prevent oxidation of the ferrous iron. Repeated analyses using the thiocyanate method[13] showed that in these flasks less than 3 per cent of the iron was present in ferric form even after one month's storage at room temperature

Also included in this paper is a separate study concerning the absorbability of iron from different iron compounds when given as tablets for a longer time The same general experimental design was employed using ferrous sulphate tablets as a reference in all subjects One tablet containing ferrous sulphate corresponding to 30 mg of elemental iron was given 3 times a day on alternate days These tablets were labelled with one of the radioiron isotopes On the other days the compound under study labelled with the other radioiron isotope was also given as tablets 3 times a day All tablets contained 30 mg of elemental iron and had a disintegration time of 15 minutes[14] Tablets were given between meals for 24 days as outlined in figure 1 A blood sample was drawn weeks after the last oral dose for deter

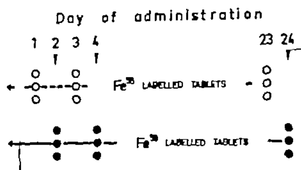


Fig 1 Dosage schedule.

mination of Fe^{55} and Fe^{59} activity Four compounds were compared in this way ferrous sulphat ferrous succinate ferrous gluconate and ferron glycine sulphate compounds. A separate study of the completeness of the exchange was made (reported below).

Preparation and labelling f iron compounds

All iron compounds used were reagent grade or prepared from reagent grade chemicals. The iron content w deter mined with the thiocyanate method using hydrogen peroxide as oxidant [12]

The compounds were labelled by isotope exchange Radsoiron with high specific act vity (not less than —3 μC μg) was added t the solutions containing non-radioactive iron[13] A complet exchange took place also with the complex iron

MATERIAL

The subjects in this study were 15 nor mal healthy volunteers (N) 54 blood donors (BD), who had served as blood donors for varying time and never received any iron supplementation. Moreover 11 patients (ID) with iron deficiency anemia or post haemorrhagic anemia without signs of continued bleeding were included in the material. The 8 subjects in whom ferric and ferrous sulphate were compared have been reported earlier[11]

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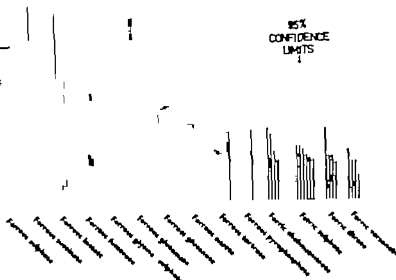


Fig. 2. Absorbability of different iron compound (solutions). Individual alues in relation t ferrous sulphate.

compounds and the trace mount f radioiron dded to the labelled compound. The variation observed is not greater than that which can be explained by analytical errors (cf. labelling of ferrous sulphate with both isotopes n previo study[18]).

B Absorption of different iron compounds (solutions)

In thi part of the study 20 mg of elemental iron as given as solution for 10 days. Ferrous sulphate wa labelled with one isotope and the compound under tudy wa labelled with the other isotope The solutions were given on alternate days for 10 days as described earlier[13] The results are given in table III and graphed in figure 2. In this figure the indivi dual absorption figures for the different compounds are graphed, expressed as a percentage of the absorption of iron from ferrous sulphate in the same indivi dual. In figure * are also graphed the 95 per cent confidence limits of the individual day to day variation of the absorption of iron from ferrou sulphate as found in a previous study in which this experimental design was used[13]

It is evident from figure that the

RESULTS

A Control of labelling procedure

The completeness of the exchange reaction when labelling the iron compounds as outlined above was tested using two isotopes of iron. The labelling of *ferrous* succinate and ferrous glycine sulphate was studied One radioiron isotope was used to label the compounds during the synthesis. The compound was purified and dissolved together with a trace amount of an other radioiron isotope The solution was given orally as a single dose and the ratio of the radioisotopes in a blood sample drawn 2 weeks later will thus be an expression for the precision of the analysis and the completeness of the exchange reaction.

Ferrous glycine sulphate was prepared in the following manner Ferrous sulphate ($FeSO_4$ $7H_2O$ pro analysi) was dissolved in boiling water under nitrogen together with Fe^{59} as ferric chloride with the same specifications as previously A warm solution containing equivalent amounts of glycine (Merck pro analysi) was added. The solution was slowly chilled under continued nitrogen bubbling and the iron compound, which was formed, was precipitated with 95 % ethyl alcohol. The compound was purified and found by analysis to be $FeSO_4$ glycine $5H_2O$ Thirty mg of iron as Fe^{59} labelled ferrous glycine sulphate was dissolved together with a tracer dose of Fe^{55} as ferric chloride (see above) This mixture was given to three subjects The results are shown in table I

Ferrous succinate was prepared in the following way to a solution of ferrous sulphate labelled with Fe^{59} a solution of equivalent amounts of sodium succinate was added The precipitate was purified and dissolved together with a trace amount of Fe^{55} (as above) This solution was given to three subjects The results are given in table II.

It is evident from table I and II that a complete exchange took place between the radioiron used in the synthesis of the

TABLE I

Labelling of ferrous glycine sulphate with two radioiron isotopes. (For details see text.)

SUBJECT	ABSORPTION (per cent)		RATIO Fe^{59} Fe^{55}
	Fe^{59}	Fe^{55}	
1 M N	3.4	3.5	1.04
2 M N	10.8	10.0	0.93
3 M D	27.1	26.3	0.97
Mean value 1.00			

TABLE II

Labelling of ferrous succinate with two radioiron isotopes. (For details see text.)

SUBJECT	ABSORPTION (per cent)		RATIO Fe^{59} Fe^{55}
	Fe^{59}	Fe^{55}	
4 M N	7.6	8	0.97
5 M D	14.4	14.3	0.9
6 M D	43.0	4[illegible].7	0.[illegible]
Mean value 0.95			

Table III Continued

IRON COMPOUND	SUBJECT	ABSORPTION (per cent)		ABSORPTION RATIO compound / reference	
		Iron compound	Reference (Ferrous sulphate)	Individual value	Mean
Ferrous citrate	39-M N	2.3	4.6	0.54	0.74
	40-M BD	12.6	21.4	0.59	
	41 M BD	21.2	23.6	0.91	
	42-M BD	29	34.4	0.84	
	43-M BD	49	62	0 [illegible]	
Ferrous tartrate	44-M N	1	4.0	0. [illegible]	0.62
	45-F N	4.	7.8	0.50	
	46-F BD	3.	8.8	0.43	
	47 M BD	14	22.3	0. 7	
	48-M BD	25.0	32.5	0.77	
	49 M BD	21.9	38.0	0.64	
Ferrous pyrophosphate	50-M BD	2.0	4.	0.44	0.59
	51 M BD	3.4	8.8	0.40	
	52-M BD	10.	18.6	0.56	
	53-M BD	18	1 [illegible]	0.77	
Ferric hobo rene trate	54-M BD	8	28.	0.30	0. [illegible]
	55-M BD	12.	27.0	0.4	
	56-F BD	15.4	31.7	0.4	
	57 M BD	12.8	32.7	0.4	
	58-M BD	9.6	38.4	0.25	
Ferric cit	59-M BD	3.6	15.	0.23	0. 1
	60-M BD	6.2	22.6	0.27	
	61 M BD	14.8	3.	0.	
	62-M BD	12.8	47	0.27	
Ferric [illegible]	63 M BD	3.6	13.6	0.2	0.2
	64 M BD	3.3	21	0.16	
	65-M BD	8.	47	0.2	
	66-M BD	10.4	42.6	0.2	

TABLE III

Absorbability of different iron compounds given as solutions containing 30 mg of elemental iron.

IRON COMPOUND	SUBJECT	ABSORPTION (per cent)		ABSORPTION RATIO compound / reference	
		Iron compound	Reference (Ferrous sulphate)	Individual value	Mean value
Ferrous succinate	7 M N	4.9	6.0	0.83	
	8-M BD	14.8	12.3	1 [illegible]0	
	9 M BD	23.8	18.0	1.32	1.23
	10-M BD	31.4	19.8	1.58	
	11 M BD	7.6	[illegible]3.1	1.20	
	1[illegible] M BD	48.[illegible]	35.0	1.[illegible]5	
Ferrous lactate	13-M N	3.5	4.0	0.89	
	14-M N	[illegible]4	3.8	1.28	
	15-M BD	[illegible]3	6.1	1.[illegible]0	1.06
	16-M N	6.3	6.4	0.98	
	17 M N	7.1	[illegible]8	0.92	
Ferrous fumarate	18-M N	4.8	4.8	1.00	
	19 M BD	1[illegible].7	18.2	0.93	
	[illegible]0-M BD	19.0	18.6	1.03	1.01
	[illegible]1 F BD	[illegible]3.5	[illegible]3.1	1.01	
	22 F BD	34.6	34	1.02	
Ferrous glycine sulphate	23 F N	4.2	3.8	1.09	
	[illegible]4-M N	10.1	9.2	1.10	
	[illegible]5 M ID	48.2	[illegible].7	0.9	1.01
	[illegible]6-M ID	41.4	46.6	0.89	
	[illegible]7 M ID	69.0	67.6	1.02	
Ferrous glutamate	[illegible]8 M BD	[illegible]1	10.0	1.16	
	[illegible]9 M BD	10.0	19.3	0.52	
	30 M BD	30.1	25.9	1.16	0.[illegible]
	31 M BD	33.4	30.2	1.10	
	3[illegible] M BD	51.1	56.6	0.90	
Ferrous gluconate	33-M BD	3.2	3.7	0.86	
	34 M BD	3.8	6.[illegible]	0.69	
	35-M BD	15.2	15.3	0.99	0.[illegible]
	36-M BD	19.1	[illegible].6	0.85	
	37 M BD	16.5	[illegible]3.3	0.83	
	38 F BD	34.9	[illegible].5	1.07	

Table III Continued

TABLE IV

Absorbability of different iron compounds, given as tablets containing 30 mg of elemental iron three times a day

IRON COMPOUND	SUBJECT	ABSORPTION (per cent) Iron compound	ABSORPTION (per cent) Reference (Ferrous sulphate)	ABSORPTION RATIO compound / reference Individual value	ABSORPTION RATIO compound / reference Mean value
Ferrous succinate	67 F N	2.	2.9	0.91	
	68-M BD	7 6	8 4	0.93	
	69-M BD	8.9	16.9	0 52	0 93
	70-M ID	21 4	17 7	1 21	
	71 M ID	23 1	28.3	0.89	
	72-M BD	23.4	28.4	0 76	
Ferrous glycine sulphate	73-F BD	10 4	10.4	1.01	
	74 F BD	9 7	10.9	0.89	0.97
	75-F BD	17	18.6	0.93	
	76 F ID	33	23 7	1 43	
Ferrous gluconate	77 M BD	5 7	8.9	0.65	
	78-M BD	8.5	11.8	0 74	0 8
	79-M BD	11	14.	0.80	
	80-M ID	20.4	22.3	0.93	

DISCUSSION

Introduction

The great number of iron compounds used to-day in the oral treatment of iron deficiency and the disparity in views regarding their therapeutic value are probably mainly a result of the difficulties in making a valid evaluation of the relative merits of different compounds.

When the same amount of iron is given, no differences in side-effect have been observed in the few published controlled studies[4]. With increasing doses of iron, more side-effects were encountered in a double-blind study in which ferrous sulphate tablets were given in doses of 30 mg of elemental iron three times a day to 100 mg three times a day[4]. Because of these facts the therapeutic value of different iron compounds will be determined mainly by their relative absorbability. In almost all previous studies on the therapeutic value of different compounds such comparisons have been based on determinations of the relative absorbability using one method or another.

The therapeutic value of iron compounds

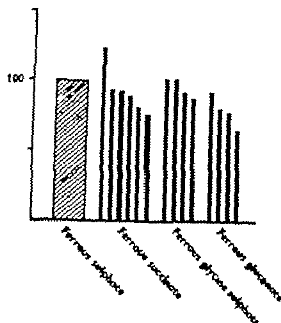


Fig 3. Absorbability of different iron compounds (tablets) Individual values in relation t ferrous sulphate

absorbability of iron from different iron compounds differs markedly The ferric compounds are less absorbed than the ferrous. Of the ferrous compounds ferrous citrate tartrate and pyrophosphate are significantly less absorbed than the other Only *one* compound ferrous succinate was more absorbed than ferrous sulphate (when solutions were compared)

The amount of iron absorbed expressed as per cent of the amount of iron administered, *is given in* the tables as Absorption (per cent) These figures are only estimates as discussed in a previous paper[12] but the errors in these estimates do not *influence the absorption ratio*

C. Absorption of different iron compounds (tablets)

In this part of the study 30 mg of elemental iron was given in tablet form 3 times a day between meals On every second day *ferrous* sulphate *tablets* were given and on the other days the tablets containing the iron compound under study were given This dosage schedule is shown in figure 1

The results are given in table IV and are graphed in figure 3 There is probably no real difference in absorbability of iron from these 4 compounds

The mean value of the absorption ratios of ferrous succinate glycine sulphate and gluconate are *not significantly* different statistically However since the variation of the absorption of iron from ferrous sulphate tablets has not been studied it is impossible to decide if the absorption of iron from any one of the three compounds *is significantly lower* than from ferrous sulphate

absorption and how much comes from stores.

4 The blood volume must be constant. To calculate the absolute amount of iron absorbed from the increase of the hemoglobin concentration the blood volume must be known.

When comparing the absorbability of iron from different iron compounds the same amount of elemental iron must be given to the different groups. The dosage schedule must be the same the disintegration time of the tablets identical etc Besides the conditions mentioned above (1—4) the *following considerations have* t be taken into account

5 The initial hemoglobin level should be bout the same in groups compared since the regeneration rate is faster when the hemoglobin level is lower Preferably the hemoglobin level hould be of the same *relative* magnitude because for example — a regeneration rat from 8 to 1 g hemoglobin per 100 ml blood will probably not be the same in t o subject in whom the final normal hemoglobin concentration is 12 and 16 g per 100 ml respectively A method for comparisons between individuals of the regeneration rate has previously been described[14]

6 The observation period has to be lim ted to the regeneration period and must be finished before some individual reaches his normal hemoglobin level.

7 The age and sex distribution should be about the same in compared groups. The degree of erythropoietic stimulation cannot be considered t be independent of sex and age as these factors are known to influence the individual normal hemoglobin concentration.

8. Besides the above mentioned known factors, which influence the regeneration rate of hemoglobin, it is important to randomize the individuals between groups to decrease the influence of other more or less unknown factors.

The great number of factors influencing a measurement of the absorption of iron from the regeneration rate of hemoglobin makes it almost impossible to draw valid conclusions of the bsorbability of different iron compounds This is especially true when materials studied by different authors are to be compared, inasmuch as such comparisons are further obscured by the fact that the method of chosing and selecting the material is very seldom described.

The selection of a suitable material and its division into comparable groups may be very difficult The great individual variation in bsorption of iron and regeneration of hemoglobin thus necessitates comparisons between fairly large groups in order to be able to draw valid conclusions of the absorbability of different iron compounds. Some of the above mentioned ideal conditions for comparative studies may be of more theoretical than practical importance However the great number of factors which have to be taken into consideration is the probable reason why so few quit adequat comparative studies of the absorbability of iron from different compounds have been published. An example of a study in which most of these factors have been considered is the on by O'Sullivan Higgins and Wilkinson[9]

The utilization coefficient is a concept

have also been assessed from toxicity determinations in animals. Acute oral iron toxicity studies in animals have occasionally served as a basis for postulating differences in tolerance of therapeutic doses of various iron compounds in man. The validity of such conclusions must be questioned since the factors determining the acute oral iron toxicity (e.g. the solubility and dissociation of an iron compound) are not identical with the factors determining the side-effects in oral iron therapy. Although the solubility of an iron compound in the gastric and/or intestinal juices may in some cases be a determining factor with respect to the acute oral iron toxicity, it does not necessarily have any connection with the side-effects seen in therapeutic dosage.

The preceding reasoning justifies the contention that the absorbability of iron from a compound is the main factor determining the therapeutic value.

It has repeatedly been shown that less iron is absorbed from ferric than from ferrous compounds. Despite numerous studies of the absorption of iron from various ferrous compounds it may be summarized that there is no evidence in the literature in favour of one compound or another. Iron compounds and preparations have often been introduced with claims of superiority with respect to absorbability. However, such claims have usually been based on studies lacking adequate controls.

There is thus a need of a critical evaluation of the absorbability of different iron compounds especially with regard to present disparities in view and the frequent occurrence of iron deficiency. A comprehensive discussion of various sources of errors in previous methods used to study the absorbability of iron compounds has not been published. Because of this such a review is included in this paper as a background for the discussion of the results obtained.

Earlier methods

In most previous studies the regeneration rate of hemoglobin during iron therapy in subjects with iron deficiency anemia has been the basis for comparisons of the absorbability of iron from different compounds[3-8].

The regeneration rate of the hemoglobin concentration will be an expression for the absorption of iron under the following conditions.

1. All absorbed iron must be utilized for synthesis of hemoglobin and must be the only limiting factor for the regeneration rate, i.e. the erythropoiesis must not be depressed by other diseases such as infections, renal disorders etc. This condition also demands that the magnitude of the oral dose be chosen in such a way that the optimal amount absorbed is not greater than that needed for an optimal erythropoiesis.

2. Extraneous loss of iron must be excluded during the period studied (e.g. bleeding, pregnancy). Moreover there should be no increased random destruction of red cells.

3. Cases with a considerable amount of iron in the iron stores must be excluded since otherwise it would be impossible to know how much iron utilized in the regeneration of hemoglobin comes from

absorption and how much comes from stores.

4 The blood volume must be constant. To calculate the absolute amount of iron absorbed from the increase of the hemoglobin concentration the blood volume must be known.

When comparing the absorbability of iron from different iron compounds the same amount of elemental iron must be given to the different groups. The dosage schedule must be the same, the disintegration time of the tablets identical etc. Besides the conditions mentioned above (1—4) the following considerations have to be taken into account

5 The initial hemoglobin level should be about the same in groups compared since the regeneration rate is faster when the hemoglobin level is lower. Preferably the hemoglobin level should be of the same *relative* magnitude because for example — a regeneration rate from 8 to 12 g hemoglobin per 100 ml blood will probably not be the same in two subjects in whom the final normal hemoglobin concentration is 1 and 16 g per 100 ml respectively. A method for comparisons between individuals of the regeneration rate has previously been described[28]

6 The observation period has to be limited to the regeneration period and must be finished before some individual reaches his normal hemoglobin level.

7 The age and sex distribution should be about the same in compared groups. The degree of erythropoietic stimulation cannot be considered to be independent of sex and age as these factors are known to influence the individual normal hemoglobin concentration.

8 Besides the above mentioned known factors which influence the regeneration rate of hemoglobin, it is important to randomize the individuals between groups to decrease the influence of other more or less unknown factors.

The great number of factors influencing a measurement of the absorption of iron from the regeneration rate of hemoglobin makes it almost impossible to draw valid conclusions of the absorbability of different iron compounds. This is especially true when materials studied by different authors are to be compared, inasmuch as such comparisons are further obscured by the fact that the method of chosing and selecting the material is very seldom described.

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The utilization coefficient is a concept

which has led to much confusion in comparative evaluations of iron compounds This coefficient was originally defined as the percentage of iron absorbed from a dose which was just sufficiently large to give a regeneration rate of hemoglobin corresponding to 1 per cent per day of the final hemoglobin mass when the severity of anemia was about 50 % [4 5 6]

However it is almost impossible to find such a dosage level for a certain individual and in almost all studies no attempts have been made to find out this minimum dosage Usually the amount of iron given was more than that which could be expected to be necessary for an optimal regeneration rate Under such conditions the utilization coefficient will be proportional to the ratio of the regeneration rate of hemoglobin to the amount of iron administered This coefficient will thus be a meaningless expression without any relation to the real absorption of the iron compounds and mainly a figure inversely related to the dose

Quite paradoxical utilization coefficient (> 100 per cent) have been reported in materials treated with small iron doses and including subjects with acute post-*haemorrhagic anemia with probably normal* iron stores Most of the iron utilized in the regeneration of hemoglobin must have been derived from these iron stores

The *reticulocyte response* to oral iron therapy has also been used to quantitate iron absorption.[4] The same general sources of error are inherent in this method. Moreover since additional factors besides iron absorption may influence the reticulocyte response this method does not offer any advantage in quantitative studies

The *plasma iron increase* after a single oral iron dose has also been used as a basis for a comparison of the absorbability of different iron compounds However the magnitude of the plasma iron increase is determined by a number of other factors besides absorption since the plasma iron level is a resultant not only of the absorp*tion rate of iron but also of the rate of* inflow of iron from storage compartments and the rate of the outflow of iron to bone marrow and stores[16 17] The greatly varying absorption on different days within the same individual[12 18] reduces further the practical possibilities of comparing the absorbability of different compounds Qualitative information of relative absorbability may be obtained if repeated studies are made in the same individual and if the material is sufficiently large However fairly small iron doses have to be given. The maximal plasma iron increase may otherwise be merely a measure of the unsaturated iron binding capacity (UIBC)[19]

Own results

From the preceding discussion of earlier methods and their sources of error it is evident that a method, in which each subject serves as *his own control* offers many advantages. The homogenity of the material with respect to severity of anemia age sex erythropoietic activity blood volume changes etc will not fundamentally affect the results employing the experimental design of the present study

The *ratio figures* obtained will be an expression for the average *absorption* and the average *utilization of the absorbed iron from* the different compound When

the absorbability of different iron compounds given in tablet form was studied, blood samples were drawn not only 2 weeks after the last oral dose but also 1 and 3 months after the last oral dose. Since the same absorption ratio was obtained on the three occasions, it can be concluded that there was no difference in utilization of absorbed iron from different compounds. The ratio figures will then be a real expression for the absorbability of iron from different compounds.

The results obtained in this study showed that much less iron was absorbed from the ferric compounds than from the ferrous compounds. The differences between the ferric compound were not great. However it is interesting to observe that the lowest absorbability was shown by ferric versenate a compound in which iron is strongly complex bound. The fact that iron was absorbed at all from this compound suggest a splitting of the complex in the gastrointestinal tract as discussed by WILL and VILTER[36] and LARSEN BIDWELL and HAWKINS[?].

As the absorbability of the ferric compound was only 1 3–1 7 of ferrous sulphat the use of these compounds in oral iron therapy cannot be considered rational. This point has been discussed in a previous paper[?].

Three of the ferrous compounds were found to be less absorbed than the others (ferrous tartrat citrate and pyrophosphat). In these compounds an appreciable part of the iron exist as complex ions. It is probable that the lower absorbability of these compounds is related to this common physico-chemical property.

Ferrous succinat was the only compound which was better absorbed than the totally dissociated ferrous sulphat. The average figure was lower than reported earlier[? ?] probably due to methodological improvements. Further studies are necessary in order to be able to interpret the observed increased absorption of iron from a solution of ferrous succinate since there is no immediate simple explanation.

Treatment with iron tablets is the usual form of oral iron therapy. For this reason a comparative study of the absorbability of iron from some compounds administered in tablet form was included in the present investigation. Four common compounds were studied and 30 mg of elemental iron (one tablet) was given 3 times a day.

Tablets were given for 4 days to reduce a possible increase in the variation of the basic absorption of iron from tablets due to variation in disintegration time of the tablets of the same compound, variation in transfer velocity of the tablets along the gastrointestinal tract, variation in interval of time between intake of tablets and meals etc. Moreover a 24 day experimental period corresponds more closely to conditions during oral iron therapy than the 10 day period used in our earlier studies. However the 4 day studies are much more difficult to perform and have thus been limited to these 4 compounds.

The absorption of iron from tablets containing ferrous succinate was not greater than from ferrous sulphate tablets, in contrast to the observed difference in absorbability of iron from solutions of these compounds. This may probably be due to the considerably lower rate of dissolution of ferrous succinate (in relation to ferrous sulphate) which may counteract

which has led to much confusion in comparative evaluations of iron compounds. This coefficient was originally defined as the percentage of iron absorbed from a dose which was just sufficiently large to give a regeneration rate of hemoglobin corresponding to 1 per cent per day of the final hemoglobin mass when the severity of anemia was about 50 %[4,5,6]

However it is almost impossible to find such a dosage level for a certain individual and in almost all studies no attempts have been made to find out this minimum dosage. Usually the amount of iron given was more than that which could be expected to be necessary for an optimal regeneration rate. Under such conditions the utilization coefficient will be proportional to the ratio of the regeneration rate of hemoglobin to the amount of iron administered. This coefficient will thus be a meaningless expression without any relation to the real absorption of the iron compounds and mainly a figure inversely related to the dose.

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Own results

From the preceding discussion of earlier methods and their sources of error it is evident that a method, in which each subject serves as his own control offers many advantages. The homogeneity of the material with respect to severity of anemia, age, sex, erythropoietic activity, blood volume changes etc. will not fundamentally affect the results employing the experimental design of the present study.

The ratio figures obtained will be an expression for the average absorption and the average utilization of the absorbed iron from the different compounds. When

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its slightly increased absorbability There were no statistically significant differences in absorbability between these four com pounds when administered as tablets

Some of the results obtained in this study support earlier observations by other authors other results are opposite to earlier findings and views It is probable that the discrepancies are mainly due to the numerous sources of error in earlier methods which have been decreased or eliminated with the double radioiron method used in the present study as already discussed.

The results show that there may be great differences in absorbability of iron from different iron compounds These differences are of such a magnitude that they have to be considered in the practical selection of an iron compound for oral iron therapy

SUMMARY

The therapeutic value of an iron com pound was concluded to be mainly related to its absorbability A double radioiron method in which each subject served as its own control, facilitated a quantitative comparison of the absorbability of diffe- rent iron compounds

A total of 14 iron compounds were stu died Ferrous compounds were clearly better absorbed than ferric Of the 4 ferric compounds studied the lowest absorption was obtained from ferric versenate a compound in which iron is strongly com plex bound

Of the 10 ferrous compounds studied a lower absorption was obtained from ferrous citrate ferrous tartrate and ferrous pyrophosphate compounds in which a considerable part of the iron is complex bound.

Slightly but significantly more iron was absorbed from ferrous succinate in relation to ferrous sulphate when the compounds were given as solutions. When these same compounds were compared in tablet form no difference was observed

No iron compound was thus found to be better absorbed than slightly soluble quite dissociated ferrous compounds (such as ferrous sulphate) under therapeutical conditions. The observed differences in absorbability of different iron compounds are of such a magnitude that they have practical importance in oral iron therapy

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INFLUENCE OF MEALS ON IRON ABSORPTION IN ORAL IRON THERAPY

By

HANS BRISE

INTRODUCTION

In order to achieve the most effective oral iron therapy it is important to study factors influencing the absorption of iron. It is known that certain components in the food may interfere with the absorption of iron (e g phosphates[1] phytates[2] eggs[3] and bread[4]) and because of that it has been suggested that iron should be taken between meals[5 8] However today it is generally recommended to take the iron doses with or immediately after meals to reduce gastric irritation[9—11]

In order to design the most rational oral iron therapy which will give the greatest absorption of iron with the fewest side-effects, it is necessary to know

( ) the relationship between the magnitude of the dose and the frequency and severity of side-effects (b) the dependence of this relationship on the way iron is given in relation to meals, and (c) the effect of meals on the absorption of iron. There is a lack of quantitative information on all three points.

The purpose of the present paper is to quantitate the effect of meals on the absorption of iron using an iron dose within the therapeutic range. Iron absorption was compared when iron was administered in two different ways in relation to meals within the same subject according to the same experimental design as previously applied[12] Iron was labelled with two different radioiron isotopes when administered in two different ways in relation to meals.

The investigation was devided into two parts In the first part (A) an iron solution was given once day for 10 days, in a fasting state or after a standardized light meal. In the second part (B) iron tablets were given three times a day for 4 days between or with the ordinary meals.

It was found that less iron was regularly absorbed when given with meals than when given in a fasting state or between meals

METHODS AND MATERIAL

The general experimental outline was the same as in previous studies using the double radioiron method[13] The details of the experimental design and the material in the two parts of this study are reported together with the results

The methods used in preparation of blood samples analysis of Fe^{55} and Fe^{59} and calculations were the same as earlier published[13,14]

RESULTS

A Effect of standardized light meal

Nine healthy volunteers were included in this study Six of these were blood donors (BD)

A solution containing 30 mg of elemental iron (ferrous sulphate) was given in the morning for 10 days On alternate days when the iron solutions were labelled with one of the two radioiron isotopes (Fe^{55} or Fe^{59}) used the solution was given after an overnight fast and no food or drink (except the usual rinse water[14]) was allowed for an additional 2 hours. On the other alternate days when the iron solutions were labelled with the other radioiron isotope a light meal was given 1/2 hour before administration of the iron solutions This meal was composed of 1 glass of milk, 1 sandwich (white bread) with cheese and 1 cup of coffee without sugar and cream A blood sample was drawn 2 weeks after the last oral iron dose for analysis of Fe^{55} and Fe^{59} The results are given in table I

A reduced absorption of iron was found in all cases when the iron solutions were given half an hour after the meal compared with the absorption in the fasting state The relative inhibiting effect of food on iron absorption seems to be greater (on a percentage basis) in individuals with lower absorption of iron. However the decrease in amount of iron absorbed when given after the meal tended to be greater at high absorption levels. In the six blood donors on an average 2 6 mg less iron was absorbed of the 30 mg iron dose when given after the meal.

B Absorption of iron from tablets given three times a day with or between meals

Four healthy blood donors (BD) were included in this study

One tablet containing 30 mg of elemental iron (as ferrous sulphate) was given 3 times a day for 24 days On alternate days when the tablets were labelled with one of the radioiron isotopes the tablets were taken together with breakfast lunch and dinner On the other alternating days the tablets

TABLE I

Influence of light meal on iron absorption. Iron administered 2 hours before and 1/2 hour after standardized meal on alternate days for 18 days in each subject

SUBJECT	ABSORPTION* (per cent)		ABSORPTION RATIO	Absorption decrease (estimated) mg F
	2 hrs before meal	1/2 h after meal	after/before	
1 M N	4.4	2.0	0.44	0.
2 F N	4.7	1.1	0.23	1.1
3 F N	5.2	2.2	0.42	0.9
4 F BD	18.6	8.6	0.42	2.6
5 M BD	18.0	15.6	0.83	0.6
6 F BD	1.6	12.7	0.41	2.3
7 M BD	24.2	13.5	0.56	3.2
8 M BD	33.9	27.6	0.80	2.1
9 M BD	43.5	20.5	0.7	3.5

ABSORPTION RATIO after before Mean value 0.5

were labelled with the other radioiron isotope and taken "between meals" according to the following schedule: the first tablet was taken in the morning after an overnight fast and breakfast was eaten 1–2 hours later, the second tablet was taken in the afternoon 1–3 hours after lunch (–4 hours before dinner), the third tablet was taken 2–3 hours after dinner. The dosage schedule is outlined in figure 1. Each subject noted in a record every day the time for the meals and the time he took the tablets. A blood sample was drawn weeks after the end of the medication. Standard solutions were prepared from 6 tablets. The total activity given to each subject was 20 μC of each isotope. The standard variation of the activity of

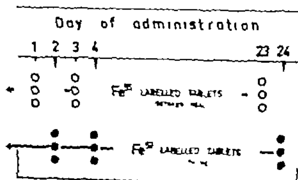


Fig. 1 Dosage schedule. One iron tablet 3 times day given between and together with meals on alternate days for 24 days and labelled with two different radioiron isotopes.

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One tablet containing 30 mg of elemental iron (as ferrous sulphate) was given 3 times a day for 24 days. On alternate days when the tablets were labelled with one of the radioiron isotopes the tablets were taken together with breakfast lunch and dinner On the other alternating days the tablets

sorbed under the two sets of condition (e.g. administration of iron with and between meals) can be quantitated. The lower absorption of iron observed, when given with or a short time after a meal, can be explained both by a chemical interaction of food components on iron (formation of insoluble or undissociated iron compounds as e.g. phosphates and phytates) and by a reduction of the concentration of iron by the bulk of the meal and by gastric and intestinal juices. All these factors can be expected to interfere with the absorption.

In a study on the effect of food factors on iron absorption by SHARPE, PEACOCK, COOKE and HARRIS[7] it was observed that the absorption of iron from a breakfast was only one fifth of that from a water solution, containing the same amount of elemental iron. It was concluded that medicinal iron should be more effective if administered between meals. However the total amount of iron given was only 8 mg including the iron in the food. It is impossible to know to what extent the radioiron added had exchanged with the food iron. If this exchange was incomplete the absorption of iron should have been overestimated calculated from the absorption of radioiron. Therefore when comparing the results by SHARPE et al. with the present results, the balance of evidence suggests that the reducing effect of a meal is more pronounced when a low iron dose is given than when a higher dose is given. Probably the reducing effect of a

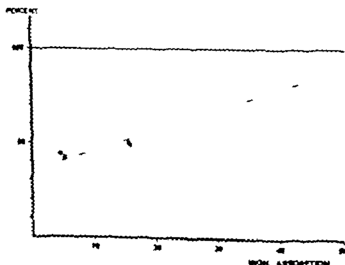


Fig. Absorption ratio as percentage (absorption after/absorption before meal) in relation to the absorption of iron in the fasting state.

Open rings (o) denote those subjects given an iron solution before and after a standardized meal. The regression line of this group is drawn as an interrupted line. Black dots ( ) denote those subjects given iron tablets with and between meals. For details see text.

TABLE II

Comparison of iron absorption at two different dosage schedules One iron tablet 3 times a day given between and together with meals on alternate days for 24 days in each subject

SUBJECT	"ABSORPTION" (per cent) between meals	with meals	ABSORPTION RATIO with meals/between meals	Absorption decrease (estimated) mg Fe
10-M BD	6 0	3 6	0 60	—
11 M BD	15 6	7 8	0 50	7 0
12 M BD	15.9	7 9	0 49	2
13-M BD	33 2	30 3	0.91	2.6

ABSORPTION RATIO with meals/between meals Mean value 0 63

the tablets was found to be $\pm 2.7\%$ The disintegration time was short (15 minutes — measured according to the British Pharmacopeia, 1958) and the same for all tablets.

The "between meal" schedule was chosen as to correspond to what might be performed in practice Each subject followed the given schedule each day

The results are given in table II It was found that there was a greater absorption of iron when taking the tablets "between meals than when taking them together with the meals. The reduction of the absorption was of the same relative magnitude in these subjects as in the group (A.) in which iron was given as a solution.

DISCUSSION

The daily iron doses recommended varies from about 100 mg to about 400 mg of elemental iron[8–11] Also when the small iron doses are given with meals, a sufficient amount of iron is usually absorbed to give an adequate hemoglobin regeneration rate e.g KERR and DAVIDSON 1958[17] Because of that it is difficult or impossible to evaluate an interfering effect of meals on the amount of iron absorbed by usual clinical observations. The purpose of iron therapy in iron deficiency is not only to normalize the hemoglobin value but also to restitute tissue iron and iron stores. Therefore the real effectiveness of oral iron therapy can not be determined only from the hemoglobin regeneration rate

With the method used in the present paper the relative amounts of iron ab-

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meal is not only related to the size of the iron dose but also to the size and composition of the meal.

In the present paper it was observed that the reduction of the absorption of iron when iron was administered together with meals seems to be less pronounced in those subjects absorbing a greater amount of iron in the fasting state This is evident from figure 2

The relationship between absorption decrease and absorption in the fasting state was studied statistically in those 9 subjects in whom a solution of iron was given before and after a standardized light meal. The regression line is graphed in figure 2 The correlation coefficient (r) was 0 73 ($p<0\ 05$) As a comparison the results from the subjects given tablets together with and between meals are also included in figure 2 (black dots)

It seems reasonable to assume that iron can be administered together with meals in patients with iron deficiency without a substantial decrease in the amount of iron absorbed.

However to be able to give a conclusive answer to the question of the most suitable dosage schedule in oral iron therapy more thorough studies are needed concerning the effect of meals on the absorption of iron at various dose levels in relation to side-effects

SUMMARY

The quantitative importance of the interference of food on iron absorption under therapeutical conditions in man was studied in two series of experiments using the double radioiron method.

In the first series the absorption of iron was compared when administered in a fasting state and when administered 1/2 hour after a light meal Iron was given in the two ways on alternate days in the same subject for 10 days and was labelled with two different radioiron isotopes On an average the absorption was reduced by half when the iron was given after the meal

In the second series two therapeutic dosage schedules were compared also using the double radioiron method. Iron tablets were given 3 times a day for 24 days, and together with meals and between meals on alternate days in the same subject On an average 40 per cent more iron was absorbed when given between meals.

It was found that the absorption decreased with 20 to 60 per cent when 30 mg iron doses were given together with meals This reduction in absorption was found to be inversely related to the absorption of iron in the fasting state

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It was found that the absorption decreased with 20 to 00 per cent when 30 mg iron doses were given together with meals. This reduction in absorption was found to be inversely related to the absorption of iron in the fasting state

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EFFECT OF SURFACE-ACTIVE AGENTS ON IRON ABSORPTION

By

HANS BRISE

INTRODUCTION

In a previous paper the absorbability of different iron compounds was compared[1]. The results indicate that it is very improbable that iron compounds can be found from which more iron is absorbed than from an easily soluble dissociated iron salt (e.g. ferrous sulphat.). The only possibility of discovering oral iron preparations, from which more iron is absorbed, seems therefor to reside in a search for substances, which in one way or another promote iron absorption as such.

Various surface-active agents have been used in pharmaceutical preparations i.a. to improve absorption. In 1954 WIESLER, BETHARD, BARKER and MORI reported that polyoxyethylene sorbitan monolaurate (Tween 20) increased the gastrointestinal absorption of iron in hamsters.

The present study is based on a double radioiron technic. The same amount of iron was given for 10 days and labelled with different isotopes when given with and without surface-active substance on alternate days, thus making each subject his own control as in previous study[4].

It was found that none of the four surface-active agents studied significantly increased the absorption of iron.

METHODS AND MATERIAL

Every morning after an overnight fast, a 25 ml solution containing 30 mg of elemental iron as ferrous sulphate was given orally for 10 days. The preparation of the solutions was the same as earlier reported[3]. Every second day the iron doses were labelled with one of the radioiron isotopes (Fe^{55} or Fe^{59}) and every second

day when a surface-active agent also was given, iron was labelled with the other isotope

The following surface active agents were used

Dioctylsodiumsulfosuccinate (*Aerosol OT* American Cyanamid Comp) was administered as a powder containing 150 mg of the substance The powder was swallowed together with the iron solution. The flasks containing the iron solution were rinsed twice and the rinse water was also taken as previously described.[4]

Polyoxyethylene sorbitan monolaurate (*Tween* 20 — Atlas Powder Comp) was taken as a solution in a separate flask containing 400 mg Tween 20 in 15 ml. The Tween 20 solution was taken immediately after the iron solution.

Sodium lauryl sulphate (U.S.P 16) was dissolved in the iron solutions given on alternate days as a dose of 200 mg

Bile acids were given in gelatine capsules. Two capsules containing 146 mg cholic acid and 37 mg dehydrocholic acid (prepared from Fellesan tablets — A.B Pharmacia, Sweden) were taken together with the iron solutions on alternate days.

A blood sample was drawn 2 weeks after the last oral dose and the ratio of the absorption of iron when given with and without a surface-active agent was calculated as previously described [3,4]

The material in this study includes 9 healthy male subjects (N) and 16 healthy blood donors (BD) who had never received any iron supplementation

RESULTS

The results are given in table I The effect of Tween 20 was studied in 4 subjects. No increase in absorption was noted in any of these subjects.

When dioctylsodiumsulfosuccinate was given together with iron a slight increase in absorption was observed in 3 of the 6 subjects studied. The mean absorption ratio did not significantly differ from the mean absorption ratio when only ferrous sulphate was given for 10 days in 24 subjects (M = 1 01 standard error of mean ±0 02) as reported in an earlier paper[4] It is thus probable that the somewhat higher mean absorption ratio (1 07) observed in this group is due to the normal variation of the absorption and is not an effect of dioctylsodiumsulfosuccinate on the absorption.

The same is also true for sodium lauryl sulphate in which case the mean absorption ratio also was 1 07 A mixture of cholic and dehydrocholic acid was likewise found not to increase the absorption of iron in 4 subjects.

TABLE I

Effect of surface-active agents on iron absorption

Surface-active agent	SUBJECT	ABSORPTION (per cent)		ABSORPTION RATIO with/without surface-active agent	
		without	with	Individual value	Mean value
		surface-active agent			
Polyoxyethylene sorbitan monolaurate (Twe en 20) 400 mg	1 M BD	13 6	16	1 0	1 0
	2 M BD	31 5	32.	1 07	
	3 M BD	23	21	0. 4	
	4 M BD	44	46 5	1	
Dioctylsodium-sulfosuccinate (Aerosol OT) 150 mg	5 M BD	4	5	1 4	1 1
	6 M BD	13	19	1 2	
	7 M BD	20.7	20 7	1.00	
	8 M BD	28	31	1 1	
	9 M BD	22.	21	0 9	
	10 M BD	41	37	0 9	
Sodium lauryl sulphate 200 mg	11 M N	3	6.	1. 8	1 07
	12 M N	6	7	1 2	
	13 M BD	16 0	16.9	1 00	
	14 M BD	29 1	23	0.80	
Cholic acid 140 mg and Dehydrocholic acid 37 mg	15 M BD	11	11	1 2	1.02
	16 M BD	17	16	0 9	
	17 M BD	21	22.	1	
	18 M BD	33	35	1	

DISCUSSION

The available area of absorption may considerably affect the amount of a substance absorbed. Especially this may be true for substances which normally are only partially absorbed. Therefore the administration of surface-active agents might theoretically be thought to increase the absorption of iron.

The amounts of surface-active agents given together with the iron solutions were as great as might be used therapeutically in iron tablets. It is evident from the results that it is not possible to increase the absorption of iron using these substances.

The observation by Wiesler et al [6] that Tween 20 increased the absorption of iron in hamsters is not comparable with the

day when a surface active agent also was given iron was labelled with the other isotope

The following surface active agents were used

Dioctylsodiumsulfosuccinate (*Aerosol-OT* American Cyanamid Comp) was administered as a powder containing 150 mg of the substance The powder was swallowed together with the iron solution The flasks containing the iron solution were rinsed twice and the rinse water was also taken as previously described.[4]

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A blood sample was drawn 2 weeks after the last oral dose and the ratio of the absorption of iron when given with and without a surface-active agent was calculated as previously described [4 6]

The material in this study includes - healthy male subjects (N) and 16 healthy blood donors (BD) who had never received any iron supplementation

RESULTS

The results are given in table I The effect of Tween 20 was studied in 4 subjects No increase in absorption was noted in any of these subjects

When dioctylsodiumsulfosuccinate was given together with iron a slight increase in absorption was observed in 3 of the 6 subjects studied The mean absorption ratio did not significantly differ from the mean absorption ratio when only ferrous sulphate was given for 10 days in 24 subjects (M = 1 01 standard error of mean ±0 02) as reported in an earlier paper[4] It is thus probable that the somewhat higher mean absorption ratio (1 07) observed in this group is due to the normal variation of the absorption and is not an effect of dioctylsodiumsulfosuccinate on the absorption.

The same is also true for sodium lauryl sulphate in which case the mean absorption ratio also was 1 0 A mixture of cholic and dehydrocholic acid was likewise found not to increase the absorption of iron in 4 subjects.

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EFFECT OF ASCORBIC ACID ON IRON ABSORPTION

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

The repeated observation that ferrous iron is better absorbed than ferric[1-3] lead to studies on the effect of reducing substances as ascorbic acid on the iron absorption. It was found that ascorbic acid increased the absorption of ferric iron and of food iron[2]. In studies in dogs[4] and rats it was found that the amount of iron absorbed from ferrous sulphate was significantly increased when administered together with ascorbic acid.

In human subjects a greater plasma iron increase was observed when iron was given together with a large dose of ascorbic acid. However when a quantitative method was used to determine the absorption of ferrous iron, no effect of ascorbic acid was observed if huge amounts of ascorbic acid were not used and it was concluded, that the addition of ascorbic acid to ferrous iron salts offered no practical advantage in iron therapy[1].

In a previous paper a method was described in which each subject served as his own control and a more exact quantitation of the effect of various substances on the absorption of iron was made possible[11]. A reevaluation of the effect of ascorbic acid on iron absorption using this method was considered to be important from both a theoretical and a practical point of view.

In the present paper it was shown that ascorbic acid, when given in sufficient amounts increased the absorption of ferrous iron and that the absorption promoting effect increased with increasing amounts of ascorbic acid. This effect is probably mainly exerted in the gastro-intestinal lumen inasmuch as intravenous administration of ascorbic acid was found to affect neither iron turnover nor iron absorption.

present findings. The animals were given large amounts of Tween 20 (5 per cent of the weight of the rations) for long time (from 8—20 weeks). EAGLE et. al. had earlier observed marked cirrhotic changes in the livers of young hamsters which were fed polyoxyethylene derivatives for longer periods of time.[5] In the study by WISSLER et al. early cirrhosis was also observed in occasional livers. It is thus probable that the increased iron absorption in the hamsters may have been due to some toxic action of Tween 20 and was not an effect directly related to a decrease of the surface-tension of the gastrointestinal content.

From these animal studies and from the present observation that Tween 20 in smaller amounts, did not increase the absorption of iron from ferrous sulphate in humans it can be concluded that there is no rational basis using Tween 20 in pharmaceutical iron preparations. This conclusion can also be extended to the other agents studied — dioctylsodium sulfosuccinate sodium lauryl sulphate and bile acids.

SUMMARY

The effect of surface-active agents on iron absorption was studied in 18 subjects using a double radioiron method, where each subject served as his own control. No significant increase of the absorption of iron was observed with any of the compounds studied (Tween 20 dioctylsodium sulfosuccinate, sodium lauryl sulphate and bile acids)

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TABLE I

Effect of ascorbic acid orally on iron absorption. Different amounts of ascorbic acid were given to 30 mg of elemental iron (as ferrous sulphate).

Amount of ascorbic acid (mg)	SUBJECT	ABSORPTION (per cent)		ABSORPTION RATIO with / without ascorbic acid	
		without	with	Individual value	Mean value and standard error of mean
		ascorbic acid			
50	1 M N	3	4	0.81	0.91 ± 0.04
	2 M N	5.[illegible]	5.[illegible]	1.03	
	3 M BD	[illegible]	[illegible]	0.[illegible]	
	4 M BD	10.[illegible]	8	0.[illegible]	
	5 M BD	11.	11.[illegible]	0.[illegible]	
	6 M BD	[illegible]	4.	0.[illegible]	
100	7 M BD	3.4	3.4	0.[illegible]	1.09 ± 0.[illegible]
	8 F BD	5.	7	1.[illegible]	
	9 M BD	5.	[illegible]	1.0[illegible]	
	10 M BD	[illegible]	7	1.[illegible]	
	11 F BD	10.	15.	1.14	
	12 M BD	21	21.[illegible]	1.0[illegible]	
200	13 M N	4.7	7	1.[illegible]	1.[illegible] ± 0.[illegible]
	14 M N	5	[illegible]	1.[illegible]	
	15 M BD	[illegible]	11	1	
	16 M BD	[illegible]	12.[illegible]	1.37	
	17 F N	[illegible]	11	1.[illegible]	
	18 F BD	10	16.	1.37	
	19 M BD	11	14	1.[illegible]	
	20 M BD	12	22	1.77	
	21 M BD	12	16.	1.30	
	22 M BD	23.[illegible]	[illegible]	0.	
	23 M BD	[illegible]	[illegible]	1.[illegible]	
	24 M BD	24.	[illegible]	1.20	
	25 M BD	[illegible]	[illegible]	0.[illegible]	
300	26 M N	4	[illegible]	1.20	1.[illegible] ± 0.[illegible]
	27 M BD	[illegible]	11	1.[illegible]	
	28 M BD	[illegible]	[illegible]	[illegible]	
	29 M BD	11	11.[illegible]	1	
	30 M BD	12	[illegible]	1.4	
	31 F BD	14.[illegible]	25	[illegible]	
	32 F BD	16	20	1.1[illegible]	
	33 F BD	21	30.[illegible]	1.[illegible]	
	34 F BD	22	[illegible]	1.[illegible]	
	35 M BD	[illegible]	31	1.[illegible]	
	36 M BD	24.[illegible]	[illegible]	0	
	37 F BD	[illegible]	46.	1.[illegible]	
500	38 F BD	11.[illegible]	[illegible]	1.67	[illegible]
	39 M BD	17	23.	1.[illegible]	
	40 M BD	18	23.	1.[illegible]	
	41 M BD		30.[illegible]	1.[illegible]	
	42 M BD	[illegible]	44.1	1.60	

METHODS AND MATERIAL

The general experimental design was the same as in previous studies[11, 12, 13]. Unless otherwise stated a ferrous sulphate solution containing 30 mg of elemental iron and labelled with radioiron was given orally every morning after an overnight fast for 10 days. Fe^{55} and Fe^{59} were used as labels on alternate days. Every second day ascorbic acid was given as tablets together with the iron solution. From analysis of Fe^{55} and Fe^{59} in a blood sample[14] drawn 2 weeks after the last oral iron dose the effect of ascorbic acid on the absorption of iron could be determined, thus making each subject his own control as in previous studies.[11]

Studies of this kind were made in 42 subjects [6 healthy volunteers (N), 1 case of pernicious anemia during treatment (PA) and 35 blood donors (BD)]. The iron doses given together with ascorbic acid were labelled with Fe^{59} in 18 subjects with Fe^{55} in 22 subjects. Twenty subjects were given ascorbic acid on the odd days of the study. The other 22 subjects were given ascorbic acid on the even days of the study.

In four additional subjects 300 mg of ascorbic acid was given intravenously instead of orally on every second day. These injections were given 5 minutes before the oral doses. These subjects were in patients without any known hematological disorder, infection, liver or renal disease. The effect of ascorbic acid on plasma iron turnover was studied in one female and one male healthy medical student.

RESULTS

I. EFFECT OF ASCORBIC ACID ORALLY ON IRON ABSORPTION

The results are summarized in table I. The term "Absorption" means absorbed iron found in the estimated red cell mass two weeks after the last oral iron dose. The figures for absorption are only given to facilitate comparisons between individuals. The systematic errors in the estimation of the absorption do not affect the accuracy of the ratio figures as discussed in a previous paper.[11]

It is shown in table I that more iron was absorbed when given together with ascorbic acid. However, a marked effect was observed only when 200 mg or more of ascorbic acid was given together with 30 mg of iron. There was a considerable variation in the effect of ascorbic acid between individuals. Part of this variation is necessarily related to the basic variation in absorption of iron on different days.[13] Another part of the variation may be related to a varying effect of ascorbic acid in different individuals and also in the same individual on different days.

The average increase of the absorption of iron, when the iron was given together with varying amounts of ascorbic acid, is shown in figure 1. The increase is expressed as the percentage of the absorption of iron when given without ascorbic acid.

A statistical analysis of the relationship between the dose of ascorbic acid and the increase of the absorption of iron is graphed in figure 2.

The following functional relationship was found within the domain studied.

$$y = 0.84 \log x - 0.17$$

where

y was the absorption ratio

and

x was the dose of ascorbic acid in milligrams.

The regression coefficient 0.84 was statistically significant from zero (t=5.07 df 40). The rest standard deviation was 0.23 and the correlation coefficient (r) was 0.6..

2. EFFECT OF ASCORBIC ACID INTRAVENOUSLY

a. Effect on iron turnover

In two healthy subjects 300 mg of ascorbic acid was given intravenously one hour after a single intravenous tracer dose of Fe^{59}. The studies were made in the morning after an overnight fast and blood samples were drawn at 10–15 minutes interval for 2 ½ hours. The technique and methods were the same as used by HALLBERG and SÖLVELL[16].

The results are shown in figure 3. It is evident that there was no effect on the iron turnover rate in these two normal subjects. Neither was any significant effect

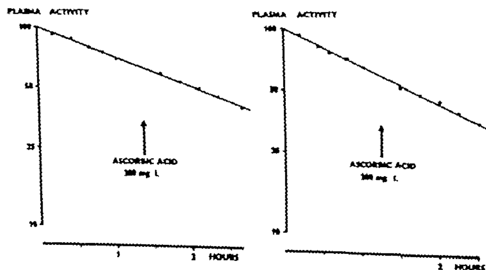


Fig. 3. Effect of ascorbic acid on plasma iron turnover rate in two subjects.

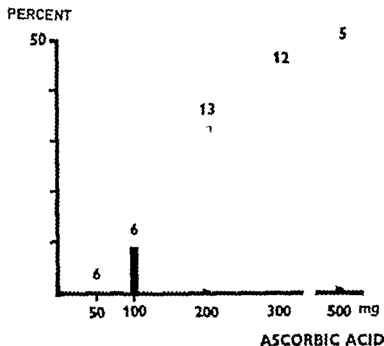


Fig 1 Average increase of iron absorption when given together with varying amounts of ascorbic acid. The figures over the bars refer to number of subjects in each group.

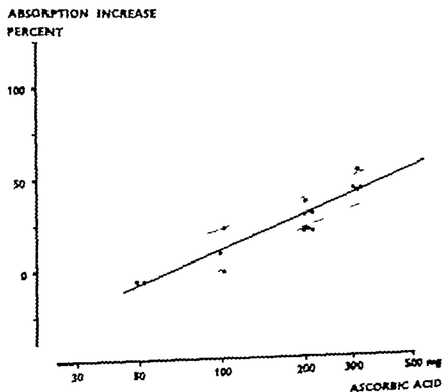


Fig 2. Relationship between amount of ascorbic acid (logaritmic scale) given together with 30 mg of iron and absorption increase.

The regression line is drawn as a solid line and the 95% confidence band for this line is marked by dotted lines. (The ordinate, absorption increase, is (r—1) 100).

The average increase of the absorption of iron, when the iron was given together with varying amounts of ascorbic acid, is shown in figure 1. The increase is expressed as the percentage of the absorption of iron when given without ascorbic acid.

A statistical analysis of the relationship between the dose of ascorbic acid and the increase of the absorption of iron is graphed in figure 2.

The following functional relationship was found within the domain studied.

$$y = 0.44 \log x - 0.17$$

where

y was the absorption ratio

and

x was the dose of ascorbic acid in milligrams.

The regression coefficient 0.44 was statistically significant from zero ($t = 5.07$ df 40). The rest standard deviation was 0..5 and the correlation coefficient (r) was 0.6..

2. EFFECT OF ASCORBIC ACID INTRAVENOUSLY

a. Effect on iron turnover

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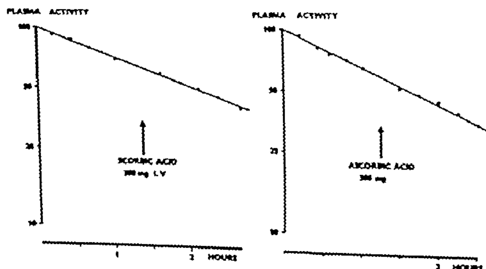


Fig. 3. Effect of ascorbic acid on plasma iron turnover rate in two subjects.

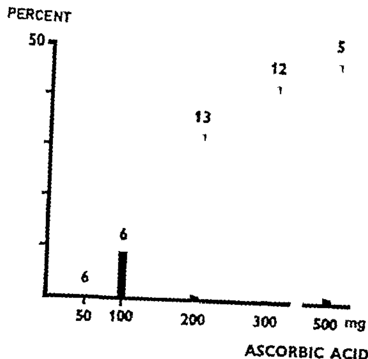


Fig 1 Average increase of iron absorption when given together with varying amounts of ascorbic acid. The figures over the bars refer to number of subjects in each group.

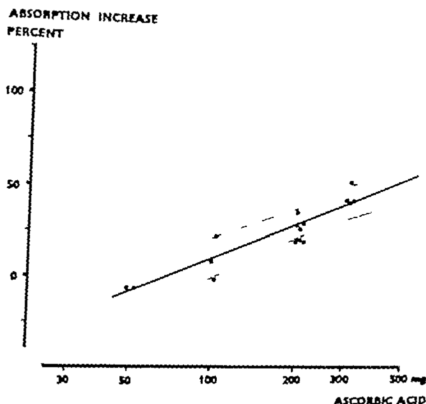


Fig 2. Relationship between amount of ascorbic acid (logaritmic scale) given together with 30 mg of iron and absorption increase.

The regression line is drawn as a solid line and the 95 % confidence band for this line is marked by dotted lines. [The ordinate absorption increase is (y—1) 100].

gastrointestinal tract. The same is also true for a great number of other ferric and ferrous compounds which may be formed in the gastrointestinal tract (e.g. phosphates). The reducing effect of ascorbic acid may help to keep the iron in the ferrous state and may thus prevent or delay a formation of insoluble or undissociated ferric compounds.

It is possible that, in addition to a reducing intraluminar effect, ascorbic acid promotes the absorption of iron by an action via internal transfer systems of iron. MAZUR et al.[7] have shown that ascorbic acid is required in addition to ATP for the incorporation reaction of transferrin bound plasma iron into ferritin. Moreover LOCHHEAD and GOLDBERG[19] have shown that ascorbic acid also increases the transfer of iron to heme biosynthesis (prot. porphyrin). From the results in this study, in which intravenous ascorbic acid influence neither the plasma iron turnover nor the absorption of iron, it can be concluded that the main effect of ascorbic acid under the conditions studied (30 mg Fe and 60–500 mg of ascorbic acid orally) is intraluminal and probably due only to its reducing action.

The absorption promoting effect of ascorbic acid observed in this study indicates that the addition of sufficient amounts of ascorbic acid to therapeutic iron doses (e.g. 200 mg of ascorbic acid to 30 mg of ferrous iron) may be of practical importance in oral iron therapy.

SUMMARY

Using a method employing two radioiron isotopes in a design in which the subject serves as his own control it has been shown,

that orally administered ascorbic acid in sufficient amounts increases the absorption of iron from ferrous sulphate and

that orally optimal amounts of ascorbic acid, when given intravenously affect neither the basal plasma iron turnover nor the absorption of iron.

It has been concluded that the absorption promoting effect of ascorbic acid is mainly due to its reducing action within the gastrointestinal lumen preventing or delaying a formation of insoluble or less dissociated ferric compounds.

observed on the plasma iron level The plasma iron turnover was thus not affected by ascorbic acid

b Effect on iron absorption

In 4 subjects iron was given for ten days as described previously labelled with Fe^{55} and Fe^{59} on alternate days. On every second day 300 mg of ascorbic acid was given intravenously *5 minutes before* the administration of the oral dose The results are shown in table II. It is evident that there was no significant effect of ascorbic acid on iron *absorption* when given intravenously

TABLE II

Effect of ascorbic acid intravenously on iron absorption 300 mg of ascorbic acid was given minutes before the oral 30 mg iron doses (ferrous sulphate)

SUBJECT	ABSORPTION (per cent) without ascorbic acid	ABSORPTION (per cent) with ascorbic acid	ABSORPTION RATIO with without ascorbic acid
43 M N	[illegible]	[illegible]	1 01
44 F N	6 7	6.4	0
45-F N	[illegible]	6 2	1 09
46-F N	1 6	1	1 0

Absorption ratio Mean value 1 [illegible]

DISCUSSION

MOORE has clearly shown that ascorbic acid promotes the absorption of food iron[5] The present paper was thus solely devoted to a reevaluation of the effect of ascorbic acid on the absorption of ferrous iron — a question which may have practical im portance in oral iron therapy Therefore a therapeutic iron dose was given together with different amounts of ascorbic acid.

In an earlier study in which the absorption was determined on two *occasions in* the same human subject — with and without varying amounts of ascorbic acid — no conclusive increase was considered *to be obtained*[10] *The present* double isotope method[11 12 13] offered a possibility to reevaluate the effect of ascorbic acid on the absorption of ferrous iron

The results presented in this paper clearly show that ascorbic acid promoted the absorption of iron when given in sufficiently high doses, and that the effect of ascorbic acid increased with increasing doses

The great variation in absorption of iron on different days in the same individual as discussed in a previous paper[11] may be the probable reason why no significant effect of ascorbic acid could be detected *using earlier methods*[1 9]

It is very probable that the iron ion concentration in the gastrointestinal tract has a determining influence on the amount of iron absorbed as judged from other studies[14] It is a well known fact that the dissociation of ferric hydroxide i much *less than the dissociation of ferrou* hydroxide at the pH existing in the

gastrointestinal tract The same is also true for a great number of other ferric and ferrous compounds which may be formed in the gastrointestinal tract (g phosphates). Th reducing effect of scorbic cid may help to keep the iron in the ferrou state nd may thu prevent or delay formation of insoluble or undissociated ferric compounds.

It is possible that in addition to a reducing intraluminar effect, ascorbic cid promotes the bsorption of iron by an action via internal tra sfer systems of iron. MAZUR et al[17,18] have shown that ascorbic acid is required in addition to ATP for the incorporation reaction of transferrin bound plasma iron into ferritin Moreover LOCHHEAD nd GOLDBERG[19] have shown that scorbic acid also increases the transfer of iron to heme biosynthesis (prot porphyrin) From the result in this study in which intravenous ascorbic acid influence neither the plasma iron turnover nor the absorption of iron, it can be concluded that the main effect of ascorbic acid under the conditions studied (30 mg Fe and 50—500 mg of ascorbic cid orally) is intraluminal and probably due only to its reducing action.

The absorption promoting effect of ascorbic cid observed in this tudy indicates that the addition of sufficient amounts of ascorbic acid to therapeutic iron doses (e g. 200 mg of ascorbic acid to 30 mg of ferrous iron) may be of practical importance in oral iron therapy

SUMMARY

Using method employing two radioiron isotopes in design in which the subject serves his own control it ha been shown,

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It has been concluded that the absorption promoting effect of ascorbic acid is mainly due to it reducing action within the gastrointestinal lumen, preventing or delaying a formation of insoluble or less dissociated ferric compounds.

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EFFECT OF SUCCINIC ACID ON IRON ABSORPTION

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

In previous paper it was reported that there were great differences in bsorbability of different iron compounds[1] It was hypothesized that, with different iron compounds, different iron ion concentrations were obtained in the gastrointestinal tract. This hypothesis could explain the lower absorption of iron from ferric compounds and from such ferrous compounds in which a considerable part of the iron was expected to be present as complex ions in the gastrointestinal tract. However no explanation could be given for the observation that more iron was absorbed from a solution of ferrous succinate than from solutions of quite dissociated iron compounds as e.g ferrous sulphate

Because of these observations it was thought that succinic ions *per se* influenced the absorption of iron. This hypothesis was tested and turned out to be correct as shown in the present paper The present paper also includes experiments to locate and to analyse the effect of succinic ions on the absorption of iron.

METHODS AND MATERIAL

The same experimental design was used in the present study as previously described, employing two radioiron isotopes, and making each subject his own control[2] The details of the experimental procedure and the material in different parts of the present study are described together with the results in the separate sections.

The methods for preparing solutions administered orally was the same as previously described if not otherwise stated. Determinations of Fe^{55} and Fe^{59} were also made according to a method earlier published[3]

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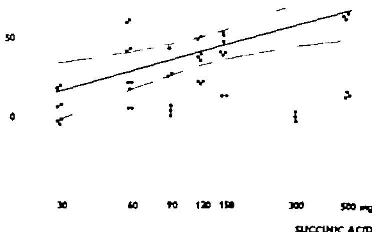


Fig 2 Relationship bet een amount of succinic acid (logarithmic scale) given together with 30 mg of iron and absorption increase

The regression line is dra as solid line and the 95% confidence band for this line is marked b dotted lines [The ordinate bsorption increase is (y—1) 100].

and M denot female and male subject respectively I 40 subjects the iron solu tions cont ining uccinic cid were la belled with Fe[59] nd in 22 subjects with Fe[55] In 23 ubject the solutions containing uccinic acid were given on odd days. The blood sample for nalyses of Fe[59] and Fe[55] wa drawn 2 weeks after the last oral iron dose as in previous studies[1]

The results are given in table I. The figures given as Absorption are not the true absorption figures as discussed in a previous paper[4] The figures mean per cent of administered iron in the estimated

RESULTS

The results of the present study are presented in six separate sections. The first one contains a study of the effect of different amounts of succinic acid on iron absorption. The following five sections contain experiments intended to analyze the mechanism of action of succinic acid

I Effect of succinic acid orally on iron absorption

Thirty milligrams of elemental iron (as ferrous sulphate) were given in a 25 ml solution also containing 10 mg ascorbic acid and 4 g sucrose The solutions were given for 10 days in the morning after an overnight fast Iron was labelled with Fe[illegible] and Fe[illegible] on alternate days Every second day when iron was labelled with one of the isotopes the solution also contained succinic acid (pro analysi, Merck Darmstadt) in amounts from 30 to 500 mg

This study included 81 subjects—13 healthy volunteers (N) and 68 healthy non anemic blood donors (BD) who had served as blood donors for varying time and who had never received any iron supplementation In table I the letters F

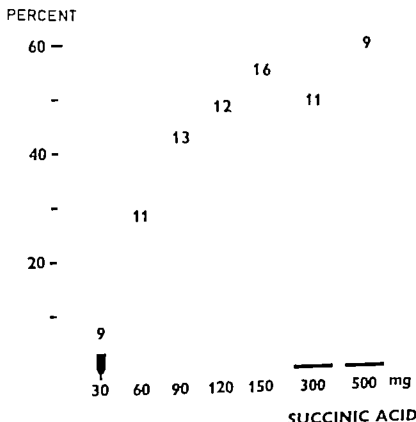


Fig 1 Increase of iron absorption by succinic acid. Each bar shows the mean value obtained from the number of subjects given above the bar

Table I Continued

Amount of succinic acid (mg)	SUBJECT	ABSORPTION* (per cent)		ABSORPTION RATIO with / without succinic acid	
		without	with	Individual value	Mean value and standard error of mean
		succinic acid			
	41-M BD	8.5	17.1	2.01	
	42-M-BD	15.6	18.5	1.19	
120	43-F BD	21.6	29.7	1.37	
	44-M BD	22.4	28.6	1.50	
	45-M BD	20.2	37.6	1.83	
	46-M N	2.2	7.2	2.23	
	47-M BD	3.4	6.4	1.89	
	48-F N	4.0	4.0	1.00	
	49-M BD	5.4	9.4	1.72	
	50-M BD	6.5	8.9	1.37	
	51-M BD	7.7	15.3	2.00	
	52-M BD	8.1	12.0	1.49	
150	53-M BD	8.1	9	1.11	1.52±0.13
	54-M BD	8.3	11.5	1.36	
	55-M BD	12.0	18.7	1.5	
	56-M BD	16.2	23.2	1.33	
	57-M BD	18.5	18.3	1.02	
	58-M BD	20.9	25	1.23	
	59-M-BD	21.7	31.	1.45	
	60-M BD	23.5	33.4	1.30	
	61-M BD	28.2	31.2	1 1	
	62-M-BD	4.6	9.2	2.00	
	63-M-BD	5.7	9.3	1.63	
	64-M BD	5.9	8.4	1.43	
	65-M-BD	9.0	21.1	2.35	
	66-M-BD	9.8	20.6	2.10	
300	67-M-BD	11.1	11.2	1.01	1. ±0.15
	68-M-BD	13.3	17.5	1.33	
	69-M-BD	15.2	17.7	1.16	
	70-M-BD	15.8	15.5	0. 9	
	71-M-BD	16.1	18.9	1.17	
	72-M-BD	24.6	22.5	0.9	
	73-M BD	8.	17.2	2.01	
	74-M BD	6.7	10.8	1.6	
	75-M-BD	10.3	16.7	1.62	
	76-M BD	10.9	12.1	1.11	
500	77-M BD	11.1	19.7	1.77	1.57±0.19
	78-M BD	11.8	18.5	1.59	
	79-M BD	12.7	14.0	1.10	
	80-M BD	20.2	22.6	1.12	
	81-M-BD	20.	28.1	1.40	

TABLE I

Iron absorption from 30 mg of iron as ferrous sulphate with and without different amount of succinic acid orally

Amount of succinic acid (mg)	SUBJECT	"ABSORPTION" (per cent) without succinic acid	"ABSORPTION" (per cent) with succinic acid	ABSORPTION RATIO with / without succinic acid: Individual value	ABSORPTION RATIO with / without succinic acid: Mean value and standard error of mean
	1 M BD	5.2	5.0	0.96	
	2 F BD	6.1	7.1	1.17	
	3-M BD	7.0	7.7	1.10	
	4-M BD	8.4	7.1	0.85	
30	5-M BD	10.7	11.3	1.05	1.03±0.04
	6-M BD	15.5	16.6	1.06	
	7 M N	18.0	17.0	0.94	
	8-M N	18.0	21.2	1.18	
	9 M BD	27.1	6.4	0.97	
	10-M N	5.8	6.1	1.04	
	11 M N	6.0	4.9	0.82	
	12 M BD	7.0	9.7	1.40	
	13 M BD	12.3	14.8	1.20	
	14 M BD	13.8	14.3	1.04	
60	15-M BD	18.0	23.6	1.32	1.25±0.0
	16-M BD	19.7	22.8	1.16	
	17 M BD	19.8	31.4	1.59	
	18-M BD	21.0	33.5	1.59	
	19-M BD	23.1	27.6	1.20	
	20-M BD	29.2	41.3	1.41	
	21 M N	3.2	4.2	1.31	
	22 M BD	4.5	8.2	1.80	
	23-M N	5.2	6.5	1.25	
	24-M N	5.3	5.5	1.05	
	25-M BD	7.1	9.9	1.41	
	26-M BD	7.8	12.6	1.62	
90	27 M BD	10.0	9.9	0.99	1.39±0.06
	28-M BD	10.2	12.6	1.24	
	29-M BD	11.0	18.7	1.69	
	30-M BD	11.6	21.0	1.80	
	31 M BD	14.6	19.0	1.30	
	32-M BD	15.9	22.0	1.49	
	33 M BD	26.6	27.3	1.02	
	34-M N	2.5	4.3	1.69	
	35-M N	5.0	7.4	1.48	
	36-M N	5.1	10.7	–.08	
120	37 M N	6.0	7.2	1.20	1.46±0.10
	38-M BD	8.0	10.8	1.36	
	39-M BD	8.0	11.7	1.47	
	40-M BD	8.4	8.2	0.98	

Table I Continued

turnover of iron e.g increased erythropoiesis, may also increase the absorption of iron[4,6]

In order to be able to analyze further the effect of succinic acid on iron absorption, it is thus necessary to know its effect on iron turnover

In two healthy volunteers a tracer dose of Fe^{59} (3—4 μC Fe^{59}) was given intravenously after an overnight fast. The details of the method were the same a those described by HALLBERG and SÖLVELL. Blood samples were drawn at intervals of about 15 minutes. After one hour 200 mg of succinic acid was given orally in a 25 ml solution.

The results were identical in both subjects. As shown in figure 3 there was no effect on the iron turnover rate Four plasma iron determinations were made during the study in each individual. Since there was no change in the plasma iron level during the study it can be concluded that oral administration of succinic acid does not influence plasma iron turnover

IV Effect of some related organic acids on iron absorption

In theory one possible mechanism of action of orally administered succinic acid on iron absorption might be a buffering action on the gastrointestinal content. With this in mind the effects of some related acids were studied. Most of the acids studied were among those which are integral parts of intracellular metabolic processes. The reason for this selection will be discussed later

The general experimental design was the same as in previous sections. A solution of ferrous sulphate containing 30 mg of elemental iron, labelled with Fe^{55} and Fe^{59} on alternate days, was given every morning for 10 days. On alternate days when the iron was labelled with one of the isotopes one millimol of acid was given in the solution (e.g 146 mg α-ketoglutaric acid).

Thirtyfive subjects were included in this study Twenty of these subjects were blood donors.

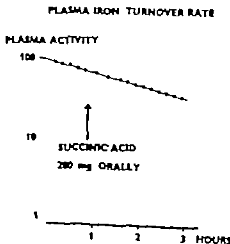


Fig 3. Plasma iron turnover rate before and after 200 mg of succinic acid orally

red cell mass 2 weeks after the last oral iron dose The systematic errors affecting these figures do not influence the absorption ratio figures. The figures for "Absorption" are only given to characterize the subjects and to facilitate comparisons between individuals

A significant effect of succinic acid was observed when 60 mg or more were added *to the solutions The mean values graphed* in figure 1 indicate that with increasing amounts of succinic acid more iron was absorbed

The following functional relationship was found within the domain studied.

$$y = 0\ 4\ \log x + 0\ 56$$

where

y was the absorption ratio and
x was the dose of succinic acid in milli grams

The regression coefficient 0 4 was statistically significant different from zero (t = 3 10 df 70) The rest standard *deviation was 0 40 and the correlation* coefficient (r) was 0 30

The observed data and the regression line are shown in figure 2

II Effect of succinic acid intravenously *on iron absorption*

In an attempt to locate the effect of succinic acid on iron absorption the acid was *given* intravenously instead of orally together *with the oral iron doses on the alternate days.*

In 5 normal subjects a ferrous sulphate solution containing *30 mg of elemental* iron labelled with Fe[illegible] and Fe[illegible] on alternate days was given *orally for 10 days as*

TABLE II

Iron absorption from 30 mg of iron as ferrous sulphate with and without 150 mg of succinic acid administered intravenously

SUBJECT	ABSORPTION (per cent) without succinic acid	ABSORPTION (per cent) with succinic acid	ABSORPTION RATIO with/without succinic acid
82 F N	3 3	4 0	1 2
83 F N	4	7 0	1 ȝ1
84-F N	5.1	5 4	1 0ȝ
85-M N	11 3	18 8	1 66
86-M N	13 8	16 3	1 0
Mean value 1 33			

described in section I On alternate days, when the iron was labelled with one of the isotopes 150 mg of succinic acid was given *slowly intravenously starting 5 minutes* before the administration of the oral iron dose

The pH of the solution given intravenously was adjusted to 7 with sodium hydroxide and the solution contained 30 mg succinic acid and 6 mg sodium chloride per ml

The results are shown in table II In 4 of the 5 subjects a *significantly higher* absorption of iron was observed when succinic acid was administered intravenously

III Effect of succinic acid orally on iron turnover

It has been observed that there is a close relationship between iron absorption and *iron turnover Any factor increasing the*

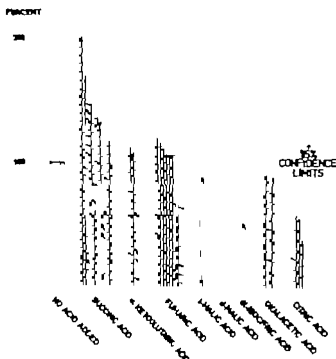


Fig. 4. Effect of some organic acids orally on iron absorption. Each acid is given in an amount of one millimol.

The results are given in table III and are graphed in figure 4.

It is evident from table III that none of the acid studied promoted the absorption of iron. On the contrary administration of citric acid reduced the absorption of iron probably because complex ferrous-citrat ion were formed.

The difference between these acids and succinic acid (one millimol being about 1?0 mg) is clearly shown in figure 4. The 95 per cent confidence limit of individual values drawn in the figure were obtained in previous study[8].

V. Effect of succinic acid on intestinal motility

Changes in intestinal motility may change the absorption from the gastro-intestinal tract. Because of this the effect of succinic acid was tested on intestinal motility.

This investigation was carried out with a method devised by KEWENTER and KOCK (For details see reference).

Three patients without current gastro-intestinal disorders were investigated, one of them had been subjected to a Bilroth II resection some years previously.

TABLE III

Iron absorption from 30 mg of iron as ferrous sulphate with and without some organic acids in amounts of one millimol.

ORGANIC ACID	SUBJECT	ABSORPTION* (per cent) without organic acid	ABSORPTION* (per cent) with organic acid	ABSORPTION RATIO with/without organic acid: Individual value	ABSORPTION RATIO with/without organic acid: Mean value
α Ketoglutaric acid (146 mg)	95-M BD	5.7	6.0	1.06	
	96-M BD	10.7	11.2	1.05	
	97-F BD	12.6	13.6	1.08	1.11
	98-F BD	19.1	21.3	1.12	
	99-F BD	4.1	20.1	1.25	
Fumaric acid (116 mg)	100-M N	3.5	3.7	1.06	
	101-M BD	4.3	5.5	1.29	
	102-M N	4.8	5.1	1.07	
	103-M BD	6.5	7.8	1.20	
	104-M BD	7.2	7.6	1.06	
	105-M N	8.0	7.6	0.94	1.08
	106-M BD	19.4	17.7	0.91	
	107-M BD	22.3	24.7	1.11	
	108-F BD	22.7	23.3	1.03	
	109-M BD	23.1	26.8	1.16	
	110-M BD	23.2	24.7	1.06	
l Malic acid (134 mg)	111-M N	4.8	4.2	0.88	
	112-M N	7.0	7.0	1.00	0.89
	113-M N	7.4	5.8	0.79	
d Malic acid (134 mg)	114-M N	2.7	1.8	0.66	
	115-F N	7.0	5.7	0.81	0.88
	116-M BD	13.5	16.0	1.18	
dl Isocitric acid (192 mg)	117-M N	7.0	6.7	0.96	
	118-F N	7.3	6.3	0.86	0.87
	119-F N	9.4	7.6	0.80	
Oxalacetic acid (132 mg)	120-M N	3.1	2.6	0.84	
	121-M BD	29.6	27.4	0.93	
	122-M BD	31.6	28.0	0.89	0.89
	123-M BD	34.9	31.3	0.90	
	1 4-M BD	40.3	36.2	0.90	
Citric acid (192 mg)	125-M N	4.9	1.9	0.39	
	126-F N	15.2	8.7	0.58	
	127-F N	18.1	10.1	0.56	0.62
	128-M BD	25.2	20.2	0.81	
	129-M BD	35.7	27.3	0.76	

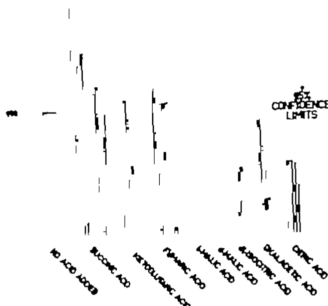


Fig. 4. Effect of some organic acids orally on iron absorption. Each acid is given in an amount of one millimol.

The results are given in table III and are graphed in figure 4.

It is evident from table III that none of the acid studied promoted the absorption of iron. On the contrary administration of citric acid reduced the absorption of iron probably because complex ferrous-citrate ions were formed.

The difference between these acid and succinic acid (one millimol being about 1*0 mg) is clearly shown in figure 4. The 05 per cent confidence limit of individual values drawn in the figure were obtained in previous study[3].

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This investigation was carried out with a method devised by KEWENTER and KOCK (For details see reference).

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TABLE III

Iron absorption from 30 mg of iron as ferrous sulphate with and without some organic acids in amounts of one mill mol.

ORGANIC ACID	SUBJECT	ABSORPTION* (per cent) without organic acid	ABSORPTION* (per cent) with organic acid	ABSORPTION RATIO with/without organic acid: Individual value	ABSORPTION RATIO with/without organic acid: Mean value
α Ketoglutaric acid (146 mg)	95-M BD	5.7	6.0	1 04	
	96-M BD	10 7	11	1 05	
	97 F BD	12.0	13 6	1 08	1 11
	98-F BD	19 1	1 3	1.12	
	99-F BD	4 1	30 1	1 25	
Fumaric acid (116 mg)	100-M N	3.5	3.7	1 06	
	101 M BD	4 3	5.5	1 29	
	102 M N	4 8	5.1	1 07	
	103 M BD	6.5	7 8	1 20	
	104-M BD	7 2	7 6	1 06	
	105-M N	8.0	8	0.94	1 08
	106-M BD	19 4	17 7	0 91	
	107 M BD	22.3	24 7	1 11	
	108-F BD	22.7	23.3	1 03	
	109 M BD	23 1	26.8	1 16	
	110-M BD	23.	4 7	1 04	
l Malic acid (134 mg)	111-M N	4 8	4 3	0.88	
	112 M N	7 0	7 0	1 00	0.89
	113-M N	7 4	5.8	0 79	
d Malic acid (134 mg)	114-M N	2.7	1 8	0.66	
	115-F N	0	5.7	0.81	0.88
	116-M BD	13.5	16.0	1 18	
dl Isocitric acid (192 mg)	117 M N	7 0	6.	0.94	
	118-F N	7 3	6 3	0 86	0.87
	119 F N	9.4	7 6	0.80	
Oxalacetic acid (13 mg)	120-M N	3.1	2.6	0.84	
	121-M BD	29 6	27 4	0.93	
	122 M BD	31 6	28.0	0.89	0.89
	123-M BD	34 9	31 2	0.90	
	124 M BD	40 3	36.2	0 90	
Citric acid (192 mg)	1 5-M N	4 9	1 9	0.39	
	126-F N	15.2	8.7	0.58	
	127 F N	18.1	10.1	0.56	0.62
	128-M BD	25.2	20.2	0.81	
	129-M BD	35.7	27 2	0.79	

—

TABLE IV

Iron absorption from 30 mg of iron as ferrous sulphate and 150 mg succinic acid with and without 200 mg of ascorbic acid.

SUBJECT	ABSORPTION (per cent) without ascorbic acid	ABSORPTION (per cent) with ascorbic acid	ABSORPTION RATIO with/without ascorbic acid
87 M BD	12.	16.4	1.36
88-M BD	21.7	28.7	1.32
89-M BD	24.4	25.4	1.05
90 M BD	33.1	38.1	[illegible]

Mean ahon 1.23

TABLE V

Iron absorption from 30 mg of iron as ferrous sulphate and 200 mg ascorbic acid with and without 150 mg of succinic acid

SUBJECT	ABSORPTION (per cent) without succinic acid	ABSORPTION (per cent) with succinic acid	ABSORPTION RATIO with/without succinic acid
91 M BD	18.4	24.2	1.32
92 M-BD	[illegible]	32.0	1.50
93 M BD	26.9	43.0	1.60
94-M BD	30.8	39.4	[illegible]

Mean ahon 1.43

This suggestion was tested using the same experimental design as employed in previous sections.

In the first study 30 mg of elemental iron (as ferrous sulphate) was given together with 150 mg of succinic acid in a solution every morning for 10 days. This amount of succinic acid was found to have an optimal absorption promoting effect. (Section I in the present paper) On alternate days, when the iron was labelled with one of the isotopes, 200 mg of ascorbic acid was also given. In this way the effect of ascorbic acid could be tested when the absorption of iron was probably optimally increased by succinic acid. The results are shown in table IV and fig 5.

More iron was absorbed in all four subjects, when ascorbic acid was given. However a statistically significant increase was obtained only in two of the four subjects.

In the second study the iron was given together with 200 mg of ascorbic acid on all 10 days. On alternate days 150 mg of succinic acid were given.

The results are given in table V and figure 6 A significant increase in the absorption of iron was observed in all four subjects. This increase was of the same magnitude as that observed when succinic acid alone was given as an absorption promoter (section I).

A soft plastic catheter was introduced through the nose into the alimentary tract in the afternoon After an overnight fast the position of the catheter tip was determined by roentgen examination to be situated about 10 cm below pylorus. The patient was lying horizontally on the back during the intestinal motility exami nation and the basal activity of the intestine was recorded for 30 minutes. A solution of 150 mg of succinic acid, dissolved in 10 ml of water was then ad ministered through the catheter The in testinal activity was then recorded for 15 minutes

No change in intestinal motility was observed in any of the three subjects investigated after administration of the succinic acid.[1])

[1]) Thanks are due to Doctors JAN KEWENTER and NILS G KOCK of the Department of Surgery I, University of Göteborg who performed the motility tests.

PERCENT

40 –

20 –



Fig 5 Independent effect of succinic and ascorbic acid on iron absorption. Effect of ascorbic acid (200 mg) in the presence of succinic acid (150 mg)

VI. Independent effect of succinic and ascorbic acids

In a previous paper it was reported that the addition of sufficient amounts of *ascorbic acid to oral iron doses significantly* increased the absorption of iron. It was concluded that this effect was mainly related to the reducing action of ascorbic acid in the gastrointestinal lumen since no effect on iron absorption was observed when the acid was given intravenously

The probable site of action of succinic acid is within the mucosal cells as will be discussed later Reasoning from the above conclusion regarding the different sites and mechanisms of action of succinic and ascorbic acids it was thought that the acids may potentiate each other s effects.

PERCENT

60 –

30

Fig 6. *Independent effect of succinic and ascorbic acid on iron absorption. Effect of succinic acid (150 mg) in the presence of ascorbic acid (*00 mg).*

TABLE IV

Iron absorption from 30 mg of iron as ferrous sulphate and 150 mg succinic acid with and without 200 mg of ascorbic acid

SUBJECT	ABSORPTION (per cent) without ascorbic acid	ABSORPTION (per cent) with ascorbic acid	ABSORPTION RATIO with/without ascorbic acid
87 M BD	12.1	16.4	1.35
88-M BD	21.7	28.7	1.33
89-M BD	24.4	26.4	1.08
90 M BD	23.1	26.1	1.16

Mean value 1.23

TABLE V

Iron absorption from 30 mg of iron as ferrous sulphate and 200 mg ascorbic acid with and without 150 mg of succinic acid

SUBJECT	ABSORPTION (per cent) without succinic acid	ABSORPTION (per cent) with succinic acid	ABSORPTION RATIO with/without succinic acid
91 M BD	18.	24.2	1.32
92-M BD	22.0	32.9	1.50
93-M BD	26.	42.0	1.60
94-M BD	30.	39.4	1.30

Mean value: 1.43

This suggestion was tested using the same experimental design as employed in previous sections.

In the first study 30 mg of elemental iron (as ferrous sulphate) was given together with 150 mg of succinic acid in a solution every morning for 10 days. This amount of succinic acid was found to have an optimal absorption promoting effect. (Section I in the present paper) On alternate days, when the iron was labelled with one of the isotopes, 200 mg of ascorbic acid was also given. In this way the effect of ascorbic acid could be tested when the absorption of iron was probably optimally increased by succinic acid. The results are shown in table IV and fig 5

More iron was absorbed in all four subjects, when ascorbic acid was given. However a statistically significant increase was obtained only in two of the four subjects.

In the second study the iron was given together with 200 mg of ascorbic acid on all 10 days. On alternate days 150 mg of succinic acid were given.

The results are given in table V and figure 6 A significant increase in the absorption of iron was observed in all four subjects. This increase was of the same magnitude as that observed when succinic acid alone was given as an absorption promoter (section I)

DISCUSSION

The starting point for the present study was the unexplained observation that more iron was absorbed from a solution of ferrous succinate than from a solution of ferrous sulphate The results in the first section of the present paper showed that the addition of succinic acid to a solution of ferrous sulphate increased the absorption of iron. These results indicate that succinic ions *per se* promote the absorption of iron because the increase was related to the amount of succinic acid added, even when more than equivalent amounts (in relation to ferrous iron) were given. The practical importance of this marked promoting effect of succinic acid on iron absorption was not studied in the present paper However preliminary studies indicate that the use of succinic acid in oral iron preparations may have great practical advantages in iron therapy[7]

In contradistinction to solutions no increased absorption of iron was observed from tablets containing ferrous succinate in relation to tablets containing ferrous sulphate as reported in a previous paper[1] As shown in table VI the solubility and rate of dissolution of ferrous succinate are considerably less than those of ferrous sulphate

At the lowest pH usually existing in the stomach (ca pH 1) both the solubility and the rate of dissolution of ferrous succinate are less than one tenth of the corresponding values for ferrous sulphate At the higher pH of the upper part of the small intestine (pH ca 5—6) the corresponding ratios are only 1:20 Table VI shows the amounts (in mg of elemental iron per ml) of ferrous succinate and ferrous sulphate which have gone into solution after different times of shaking 40 g ferrous sul

TABLE VI

Rate of dissolution of ferrous sulphate and ferrous succinate at 37° C at various pH levels expressed as g Fe dissolved in 1 ml solvent after different times See text

IRON COMPOUND	Time (minutes)	pH 1 (0 1 N HCl)	pH 5 5 (1/20 M bicarbonate-HCl buffer)	pH 7 (water)
Ferrous sulphate ($7H_2O$)	2	0.054	0.057	0 033
	5	0 063	0.057	0.068
	10	0.095	0.071	0.093
Ferrous succinate ($2H_2O$)		0.004	0.002	0.002
	5	0.003	0.004	0.004
	10	0.008	0.004	0.004
	20	0.009	0.004	0.004

phate ($7H_2O$) and 20 g ferrous succinate ($4H_2O$) respectively in 50 ml solvent at 37 C t variou pH levels.

Because the conditions for the absorption of iron are more favourable in the upper part of the gastrointestinal tract (e.g. lower pH greater area for absorption) the time factor will be of importance for the absorption of iron. The time for the disintegration of the tablets and the dissolution of the iron compound can thus be expected to influence the mount of iron absorbed. The great difference in rate of dissolution of ferrous succinat nd ferrous sulphat will thus be a probable explanation for the absence of the ex pected increased absorption of iron from ferrous succinate tablets.

The main part of the present paper consisted of studies designed to analyze the mechanism of action of succinic acid on iron absorption.

Roughly the bsorption of iron will be increased.

( ) if the concentration of iron ions in the gastrointestinal lumen is increased (or more xactly if the product of the concentration and area of absorption is increased)

(b) if the transfer of iron across the mucosal cells is stimulated or

( ) if the elimination of bsorbed iron from plasma to other sites of the body i enhanced.

The first lternative explanation for the action of succinic acid — increasing the concentration of iron ions in the gastrointestinal tract — was studied in various ways

The bsorption promoting effect of succinic acid can not be a simple acid effect, because other acids related to succinic acid did not increase the absorption of iron (section IV) Neither is it probable that succinic acid acts as a reducing agent in th gastrointestinal lumen, inasmuch as the effect of succinic acid wa not less when almost optimal amount of ascorbic acid as a reducing agent were also given together with the iron (section VI)

The result in section II that the absorption of iron was increased also when succinic acid was administered intravenously indicate that the absorption promoting effect of succinic acid cannot be due to an action on the gastrointestinal con tent (the iron ion concentration). When given intravenously the concentration of succinic acid in the gastrointestinal con tent must be much lower than when the acid is given orally In section I it was found that no effect of succinic acid was observed when doses lower than 60 mg were given orally Only 150 mg succinic acid was injected intravenously and an absorption promoting effect was evident in most cases. An increased area of bsorption is not a probable explanation for the effect of succinic acid, because no change in intestinal motility was observed as a result of oral administration of 150 mg succinic acid (section V) It can thus be concluded, that succinic acid does not exert its action through an increased iron ion concentration in the gastrointestinal content or through distribution of iron over a greater gastrointestinal surface

The two main remaining alternatives attempting to explain the promoting effect of succinic acid on iron absorption are therefore (a) an increased transfer

across the mucosal cells and (b) an increased elimination of iron from plasma. The latter was studied in section III, and *no effect of succinic acid on iron turnover* could be observed.

By a process of elimination the probable explanation for the action of succinic acid on iron absorption, will then be a direct stimulation of the transfer of iron through the mucosal cells

This interpretation of the present data fits in with recent observations suggesting that the absorption of iron is an active process dependent upon oxidative metabolism and the generation of phosphate-bound energy[9]

Succinic acid is an integral part of the citric acid cycle Addition of succinic acid can thus be expected to increase the energy available for the intracellular mucosal transfer of iron. However the observation in section IV that other acids comprised in the Krebs cycle did not increase the absorption of iron, makes it probable that succinic acid exerts its action in some other step in the intracellular *metabolism Succinic acid is for* instance also linked with the cytochrome system There may also be other steps in which the amount (or concentration) of succinic acid has a determining influence on the rate of cellular metabolism. However a further analysis necessitates the use of other methods than those employed in the present investigation.

The fact that succinic acid increases the active transport of iron across the mucosal cells suggests that the absorption of *other substances may also be increased* by succinic acid It is also possible that succinic acid is a rate limiting factor for the active transfer of some substances through other cells besides those of the gastrointestinal mucosa because the energy metabolism of all cells in the body follows the same general pathways.

SUMMARY

Succinic acid was found to increase the absorption of iron *The increase* was related to the amount of succinic acid added to the oral iron dose, (81 subjects)

The mechanism of action of succinic acid on iron absorption was studied in a series of experiments in *48* subjects.

It was concluded that the promoting effect on iron absorption was due to a direct action on the transfer of iron across the mucosal cells for the following reasons

(1) succinic acid did not affect iron turnover or intestinal motility

(2) intravenously administered succinic acid also increased the absorption of iron,

(3) other organic acids related to succinic acid (when given in equivalent amounts — one millimol) did not increase iron absorption

It is suggested that succinic acid exerts its action by increasing the intracellular mucosal metabolism It is possible that the *absorption of other* substances may also be promoted by succinic acid.

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By a process of elimination the probable explanation for the action of succinic acid on iron absorption, will then be a direct stimulation of the transfer of iron through the mucosal cells.

This interpretation of the present data fits in with recent observations suggesting that the absorption of iron is an active process dependent upon oxidative metabolism and the generation of phosphate-bound energy*.

Succinic acid is an integral part of the citric acid cycle. Addition of succinic acid can *thus be expected to increase the* energy available for the intracellular mucosal transfer of iron. However the observation in section IV that other acids comprised in the Krebs cycle did not increase the absorption of iron, makes it probable that succinic acid exerts its action in some other *step in the intra*cellular metabolism. Succinic acid is for instance also linked with the cytochrome system. There may also be other steps in which the amount (or concentration) of succinic acid has a determining influence on the rate of cellular metabolism. However a further analysis necessitates the use *of other methods than those employed in* the present investigation.

The fact that succinic acid increases the active transport of iron across the mucosal cells suggests that the absorption of other substances may also be increased by succinic acid. It is also possible that succinic acid is a rate limiting factor *for the active transfer of some substances* through other cells besides those of the gastrointestinal mucosa because the energy metabolism of all cells in the body follows the same general pathways.

SUMMARY

Succinic acid was found to increase the absorption of iron. The increase was related to the amount of succinic acid added to the oral iron dose (81 subjects).

The mechanism of action of succinic *acid on iron absorption was studied in a* series of experiments in 48 subjects.

It was concluded that the promoting effect on iron absorption was due to a direct action on the transfer of iron across the mucosal cells, for the following reasons.

(1) succinic acid did not affect iron turnover or intestinal motility.

(2) intravenously *administered* succinic acid also increased the absorption of iron

(3) other organic acids related to succinic acid (when given in equivalent amounts — *one millimol*) did not increase iron absorption.

It is suggested that succinic *acid exerts* its action *by increasing* the intracellular mucosal metabolism. *It is* possible that the absorption of other substances may also be promoted by succinic acid.

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SUPPLEMENTUM 375

GENETIC AND CONSTITUTIONAL ASPECTS OF DIABETES MELLITUS

BY

SVEN E. NILSSON



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LUND 1962

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 375

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CONTENTS

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1 INTRODUCTION

The purpose of the present investigation was to elucidate and evaluate:

1. *The hereditary background of diabetes, especially of the juvenile type*
2. *Constitutional features associated with an assumed diabetes gene.*
3. *Constitutional and environmental factors favouring the manifestation of diabetes*
4. *The effect of manifest diabetes on physical development and intellectual endowments.*
5. *The results of intravenous glucose tolerance test i various groups with different risks of being carriers of an assumed diabetes gene*

The investigation was carried out chiefly on 3 groups of young males:
- with manifest diabetes,
- non-diabetic but, owing to diabetes among their relatives, apt to be carriers of diabetes gene and
- non-diabetic, without any known heredity for diabetes.

Differences in persons with manifest diabetes compared with non-diabetic relatives of diabetics were ascribed to:
- factors favouring manifestation and thought to be observable particularly during premorbid development,
- changes due to the disease

Differences found on comparing healthy relatives of diabetics and of non-diabetics were ascribed to
- factors associated directly with an assumed diabetes gene,
- inherited factors increasing manifestation among the relatives of the diabetic.

Since all males in Sweden are liable to national service a group of military conscripts of uniform age and registered on the same occasion provided a suitable material for the investigation. On induction all members of an annual class and residing in one and the same military registration district were therefore given a written inquiry as to whether they or any of their relatives had or had had diabetes.

An advantage of such a group is that it is representativ of a definite geographical region and is not biased by various non-geographical selection factors, always diffi cult to evaluate. Since the subjects were of uniform age, possible sources of error involved by age-differences were avoided. On the other hand, this uniformity of age meant limitation of the universality of the material. This limitation could, however be overcome to some extent by collecting data on the earlier development of the probands and the state of their relatives, the latter being speciall valuable for the investigation when diabetics.

The weights and heights of the probands t birth and during school age were obtained partly from the records at depart ments of obstetrics and from the records kept by school-nurses.

II MATERIAL

The material comprised 2 series of young males.

Series A — young diabetic males

Series B

Br — young male relatives of diabetics

Bc — controls

SERIES A

119 males with manifest diabetes mellitus, born in the years 1934–1942 and representing about 90 % of all diabetics in this age-group, who at the time of the investigation were living in Scania, the southernmost province of Sweden with 880 983 inhabitants on Jan. 1, 1958. Scania is divided into two administrative areas, namely Kristianstads län with 256,040 inhabitants (1958) occupying the north-eastern part, and Malmöhus län with 611,937 inhabitants (1958) occupying the south-western part of the district and containing Malmö (217,330 inhabitants), the largest town in Scania. The social structure is elucidated to some extent by the fact that the urban population in Kristianstads län represents [illegible]2 % of its total population, as against 6[illegible] % in Malmöhus län, or 45 % if Malmö be excluded (*Statistisk Årsbok för Sverige* 1959).

The province of Scania has, in addition to university hospitals at Lund and Malmö, 6 other hospitals with special departments of internal medicine. In view of the organization of the medical service in this district, it was assumed that most diabetics of the ages in question were on the registers of the above-mentioned hospitals, or of a few other physicians particularly interested in the disease. These registers were therefore searched for male diabetics born in the years 1934–1942. The number found was then compared with the number of diabetics in the military registers, where notes are made of subjects suffering from diabetes, exempting them from military service.

The differences between the military registers and the hospital records, which are apparent from Table 1, are due in part to removal of probands during the interval between registration, when the probands were 18–19 years of age and when exemption was usually granted, and the collection of data for series A (July 1959–Feb. 1960).

It is clear however from Table 2 that no difference in weight, height or result of intelligence test was found between members of series A and those diabetics noted in the military registers but not belonging to series A, which could consequently be considered representative of the age-class investigated.

Certain data on persons immediately following the members of series A in the military and school registers were selected for comparison with equivalent data on the probands.

SERIES B

18-year-old males belonging to the 6th military registration district, and registered

The 18-year-old probands proved to possess but meagre knowledge of various familial details of relevance. In contrast, the middle-aged mothers of these probands, all of whom received written inquiries, gave extensive information on illnesses the members of the family had had, on the birthweights of their children, on their own weights and on the weights of their husbands, etc.

A decisive advantage of a series consisting of conscripts is also the ready availability *of age-matched controls.*

After collection of this group of 18-year old conscripts in whom manifest diabetes might be expected, on genetic grounds, to develop more often than in the population in general a glucose tolerance test was done on about half of the probands and their controls, to ascertain any correlation between the risk of manifest diabetes, as judged from the results of glucose tolerance tests and from genetic calculations.

Since the penetrance at 18–19 years of age is low the group of diabetics was extended to include diabetics in 2 military registration districts and born in the years 1931–1942. This implies a difference between the groups, which was, however counteracted by the use of data noted in the military record sheets of the diabetics at the time of induction and by treating the probands from the two military registration districts separately

As the material lent itself well to an all round examination, numerous data considered useful in the elucidation of somatic, mental and hereditary aspects were collected and, when possible, arranged in such classes as to fit a Gaussian curve. Correlations between all factors studied were then calculated with an electronic data machine. Diabetics and non-diabetics were treated separately

The correlation coefficients found for the diabetics and for the non-diabetics *were compared, and those coefficients* considered of relevance were analysed further

The description of the results includes chiefly those coefficients and other values bearing directly on the problems outlined above. Some supplementary coefficient tables demonstrating various methodological problems will also be given in Appendix III.

Any attempt to give a detailed survey of the voluminous literature on the aspects pertinent to the present investigation would be futile It was therefore decided to limit references to a reasonable minimum and to draw generously on recent monographs by DANOWSKI (1957), JOSLIN *et al.* (1959) and WILLIAMS *et al* (1960)

A brief survey of previous relevant investigations is given in the respective chapters.

I thank fil. kand. Torgil Ekman for kind help with the treatment of the data in the electronic data machine.

II MATERIAL

The material comprised 2 series of young males:

Series A — young diabetic males

Series B

Br — young male relatives of diabetics

Bc — controls

SERIES A

119 males with manifest diabetes mellitus, born in the years 1934—1942 and representing about 90 % of all diabetics in this age-group, who at the time of the investigation were living in Scania, the southernmost province of Sweden with 869,983 inhabitants on Jan. 1 1958. Scania is divided into two administrative areas, namely Kristianstads *län* with 258,046 inhabitants (1958) occupying the north-eastern part, and Malmöhus län with 611,937 inhabitants (1958) occupying the south-western part of the district and containing Malmö (217,530 inhabitants), the largest town in Scania. The social structure is elucidated to some extent by the fact that the urban population in Kristianstads *län* represents [illegible]2 % of its total population, as against 67 % in Malmöhus *län*, or 43 % if Malmö be excluded (*Statistisk Årsbok för Sverige 1958*).

The province of Scania has, in addition to university hospitals at Lund and Malmö, 6 other hospitals with special departments of internal medicine. In view of the organization of the medical service in this district, it was assumed that most diabetics of the ages in question were on the registers of the above-mentioned hospitals, or of a few other physicians particularly interested in the disease. These registers were therefore searched for male diabetics born in the years 1934—1942. The number found was then compared with the number of diabetics in the military registers, where notes are made of subjects suffering from diabetes, exempting them from military service.

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Certain data on persons immediately following the members of series A in the military and school registers were selected for comparison with equivalent data on the probands.

SERIES B

18-year-old males belonging to the 6th military registration district, and registered

Table 1 Composition of Series A

	Belonging to		Total
	6th mil. reg. distr	th mil. reg. distr	
Personally examined	60	49	109
Not personally examined	7	3	10
Total	67	52	119
Noted as diabetic in military register	71	68	139
Noted in military register but not in series A	15	20	35
In series A but not in military register	11	4	15

Table 2 Data from military registration sheets on diabetics born 1934—1941 belonging and not belonging to series A

	n	Weight at inducti n (mean)	Height at induction (mean)	Mean score intell. test (mean)
Series A	93	63.2 kg. SD 6.2	173.8 cm. SD 7.3	5.24 SD 1.95
Noted as diabetic in military register not belonging to series A	35	61 2 kg.	173.2 cm.	5.24

in the autumn of 1959 The 6th military registration district consists of the northern part of Scania, its southern boundary divid ing the province into 2 parts, each con taining about half of the population.

According to Swedish law every male is obliged to present himself for induction in the year he becomes 18 years of age and to do his military service the following year

Under special circumstances, a man may appear for induction somewhat earlier or later Of those who were registered rela tively early or late, however only those were included in the present investigation who were registered the year before or the year after they filled 18 years, which was 0.5 % of the whole series B.

Personal appearance is obligatory for all men called up, unless they can produce a medical certificate that they are suffering *from some disease rendering them incap*able of doing their national service. Therefore 20 (0 ¯ %) of the men in the year *class did not present themselves. These* consisted of persons with mental disorders, epilepsy severe orthopaedic diseases, or with poor vision, etc.

Diabetes is regarded as a ground for exemption from military service, although most diabetics present themselves for examination

Series B was divided into two subseries Br and Bc.

Series Br consisted of conscripts with diabetic near relatives, *i.e* sibs, parents

Table 3. Composition of series B.

Number of men liable to registration in the 6th military registration district 1959			2,834
Registered elsewhere			155
Exempted on medical grounds and not presenting themselves for registration			20
Total			2,659
Br			
Diabetes in family reported on registration	243		
Erroneous report on heredity — excluded	11		
Erroneous report discovered by answers given by mothers — transferred to Bc	4	228	
Persons originally assigned to Bc but, after report of diabetes heredity from mothers, transferred to Br		9	
Total Br		237	
Bc			
Recruits with Army number immediately after those of the Br probands	243		
Transferred to Br — cf. above	9	234	
Transferred from Br to Bc — cf. above.		4	
Total Bc		238	
Minus unexamined seamen		8	
Number of diabetics in year class		6	

ths of parents, grandparents and 1st cousins. As controls of series Br use was made of conscripts registered immediately after the Br probands. The control series was called Bc.

The composition of series B is given in Table 3.

In May June, 1960, 200 subjects out of 478 registered in B were examined by the author at their barracks, where supplementary data were obtained and glucose tolerance tests were performed.

The following groups were not included in that personal examination:

I. (154 persons). Conscripts whose national service was postponed until 1961 because of special circumstances, i.e. recent lowering of the age for national service and unusually large number of men being liable to service that year.

II. (44 persons) Conscripts called up later in 1960 i.e. those belonging to the navy and the coast artillery as well as some others with special training.

III. (32 persons). Conscripts of units stationed far away from the investigation laboratory (generally more than 250 kilometres).

IV. (18 persons). Exempted by disease at military registration. Mostly persons with orthopaedic diseases or poor vision.

V. (10 persons) Conscripts who had been enlisted but who could not be examined owing to some accidental disease etc.

VI. 8 persons belonging to series Bc who did not appear for induction, all of them were seamen.

In an attempt to form a rough opinion of the effect of these exceptions on the results, the mean weights and heights of these groups were calculated and compared with the values for the whole series and the group personally examined by the author(See Appendix I, Table 1)

Groups IV and V seem to differ to some extent, but since these groups were small, any influence they might have exerted was considered negligible.

Certain differences may be suspected between series Br and Bc in addition to diabetic relationship Assignment to series Br requires a certain knowledge of relatives so that persons belonging to groups with better knowledge of their relatives may be over represented in this series. This bias will, however be negligible concerning those probands assigned to series Br because of diabetes in their brothers, sisters or parents

In order to check whether differences in composition between series Br and Bc were due chiefly to a preponderance of diabetes in certain sociologic groups or to differences in knowledge of relatives, the probands in series Br and Bc were classified according to occupation. Those probands in Br with diabetic parents or sibs were taken together as a separate group The distributions are given in Appendix I, Table 2, which shows that series Br contained a relatively high percentage of students, while Bc contained a relatively high percentage of industrial workers The distribution of probands with diabetes in close relatives did not differ appreciably from that in the entire Br series, which suggests that the differences found between Br and Bc were due to an over-representation of diabetes in certain sociologic groups rather than to differences in knowledge of relatives.

It is also clear from Appendix I Table 2, that the distribution according to occupation of those examined by the author personally was somewhat different from that of those who were not examined personally Thus, the latter group contained a larger percentage of students who had been granted postponement of their national service on educational grounds.

In order to obtain a measure of the relation between a given occupation and physical development, the mean weights and heights of the men at the time of induction were also calculated. This made it possible to correct these values in series Br to match the composition of series Bc.

Since series B is fully comparable with only those probands of series A who belonged to the 6th military registration district, it was considered desirable to compare the sociologic composition of series A and B after division of series A according to the military districts to which the probands belonged. The probands were then grouped according to the occupations of the fathers since the probands occupations in series A and B were not fully comparable owing to differences in age levels. The results of the comparisons are summarized in Appendix I Table 3 which shows that the differences found were not significant and could be ignored on appraisement of data influenced by sociologic factors.

III METHODS

PHYSICAL EXAMINATION

Collection of data on weight and height
- At birth
- At 13 years of age
- At about 18 years of age

Body composition
- Skeleton
- Muscles
- Fat tissue

Blood pressure

Pulse rate

COLLECTION OF DATA ON WEIGHT AND HEIGHT

At birth. Of all children born in 1941 in Kristianstads län, in Malmohus län and in the town of Malmö, 72 %, 90 % and 96 % (*Sveriges Officiella Statistik 1941*), respectively were born in hospitals, where weights and lengths of the newborns were regularly noted. The weights and lengths of the probands were therefore collected as far as possible from the hospital records. However since many of the probands were not born in Scania and since some could not be traced in the hospital records, information was obtained in this way on only 53 % of the probands in series B and 41 % in series A.

In addition, information on the weights of the probands and their sibs was obtained from their mothers. These data agreed surprisingly well with those collected from the hospital records, the mean weight and range being just the same. As to the 167 probands of series A and B whose birthweights were obtained from the mothers as well as from the registers of the obstetric departments, the same mean value of 3.58 kg. was found for both groups. In 54 cases the weights reported were the same as those noted in the hospital records in 55 the difference was at most 100 g in 28 the reported weights were more than 100 g. higher and in 30 more than 100 g lower, than those noted in the records.

Information on the birthweights of the probands was thus supplemented and then covered 79 % in series B and 75 % in series A. Information was obtained of the birthweights of the sibs of 49 % of the probands in series B and of 47 % in series A.

For special purposes (see Chapter VI) information was also collected on the weights and lengths of all children born at the departments of obstetrics in Kristianstad in 1941 and in Malmö 1960

At 13 years of age At the time of the investigation school attendence was obligatory until the age of 13 years. In order to collect as large a series of children with diabetes as possible this age was chosen for the investigation. Data on 13-year-old boys were collected by written inquiries to school-nurses of the districts in which the probands had attended school.

The data were obtained from the records of the routine annual medical examination by a school-doctor or a school-

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present investigation the length of the tibia and of the radius on the right side were measured. These measurements were made according to MARTIN (1928) and LINDEGÅRD (1953, 1956). The body-height was thought to represent mainly the length factor even if influenced by the sturdiness factor and possibly by exogenic factors — e g type of work and physical training during adolescence, etc.

The growth curves for the shaft bones and the truncal height are not always parallell (STOLZ & STOLZ 1951). It is known that in the prepuberal years the legs grow faster than other parts of the body In males the legs are longer than in females because of later puberty and later closure of the epiphysis. When testosterone secretion has started it seems to initiate truncal height growth resulting in a higher sitting height of males. (For survey see TANNER, 1955).

The *sturdiness factor* of the skeleton was expressed by the right femoral condylar breadth and the right bistyloid radio-ulnar breadth measured according to MARTIN (1928) and LINDEGÅRD (1953 1956).

The factors determining condylar breadth are probably largely the same as those dictating the cross sectional area of the muscles, and muscle strength. It is possible that the breadth of the other parts of shaft bones are dependent mainly on the volume of the marrow space which seems to vary in different way (T NNER et al., 1939). Some correlation-coefficients of length- nd sturdiness-factor are given in Appendix III, T ble 3 and 4 respectively

Muscles. As mentioned bove cross-sectional areas of the muscles seem to vary with the sturdiness of the skeletal frame work (LINDEGÅRD 1953, 1956). The volume of the muscles may also be influenced by length-factor closely correlated with the length-factor of the skeleton.

I addition some special methods have been described for estimating muscularity The most valuable of these appears to be measuring muscle strength with a dynamometer (STOLZ & STOLZ, 1951), the recording being considered proportional to the total cross sectional area of all fibers of a given muscle (LINDEGÅRD, 1953).

In the present investigation the strength of the handgrip and the thrusting and pulling power of the shoulder muscles were measured according to STOLZ & STOLZ (1951). All of the dynamometric recordings were made 3 times, and the best perfor mance was noted.

The men were requested to press the dynamometer gradually *i e* not by jerks. During the measurements the persons were in the erect position with neither the arms nor the hands touching the trunk. The tests were performed at intervals of at least 3 minutes. The dynamometers used were tested gainst known weights on 3 occa sions during the investigation.

STOLZ & STOLZ (1951) found a discrepancy between the dynamometric results nd other events of adolescence in a longitudinal study in their material there appeared to be peak of strength increase in boys about 1½ years after the peak of height growth and 1 year after the peak of weight growth. It seems probable that the growth of muscle volume at this age level coincides chiefly with the increase of body weight.

The difference between growth spurt and strength spurt may be ascribed to the continuous increase of androgen secretion during the years following the period of maximal growth (TALBOT *et al.* 1943) The increase in the volume of the muscles may be due mainly to an increased activity of growth factors, g the pituitary growth hormone while the increasing strength may represent "the effect of adrenocortical and testicular hormones on the protein

nurse. As a rule, the measurements had been taken with the children stripped. Measurements were not obtainable from all of the probands because *some of them had* not been examined at 13 years and because *some of the school* records were not complete. For those born in 1934–1937 no values at all were obtainable because the records had been destroyed Data were obtained on all together 114 subjects of series B out of 209 examined personally by the author *i. e* 55 %. In series A the corresponding numbers were 48 out of 119 *i e* 40 %.

At induction The weights, heights, and circumferences of the chest noted on medical examination at induction were obtained from the military registration cards. All measurements were made with the subjects naked, and by trained personnel.

The heights and weights were also measured by the author on the occasion when other somatic measurements were made. Shoe sizes were then also noted.

Weights were given in kilograms with one decimal, with the exception of new *borns*, whose weights in the hospital records were generally given with two decimals.

Heights were given in centimetres.

BODY COMPOSITION

The skeleton, muscles and fat tissue are responsible for the major part of total body weight, and show the widest range of variation.

Judging from the few anatomical dissections on record (For survey see VON DÖBELN 1956) and data on the proportion of fat at different ages as measured densitometrically (BROZEK *et al.*, 1953, LIDI & LUFT 1961) the mean ratios between *the volumes of fat tissue skeleton* and muscle tissue in a normal 20-year old *male is roughly 1 2 4* These ratios differ with dietary habits and physical exertion, but genetic disposition is probably of great importance

Detailed analyses of the effect of different hormones on growth have been performed mainly on animals and are therefore not strictly applicable to human beings. It is evident that the hormones participating in the regulation of growth do not affect all parts of the body to an equal degree, and that the growth response of a given tissue varies with the pattern of the stimulus (RUSSELL & WILHELMI, 1958, SCOW 1959)

Various methods are available for assessment of the relative amounts of the above-mentioned types of tissue but only those that could be used in a field investigation could be considered.

In the choice of methods due attention was given to the work of LINDEGÅRD (1953, 1956) which was also partly performed on conscripts and allows of a comparison of values and correlations found in the present investigation. The terms length factor *sturdiness factor* muscle factor and fat factor in the sense used by LINDEGÅRD were *utilized for differential* somatologic classification.

Skeleton The *skeletal framework* is described by a length factor reflecting endochondral growth, *and a sturdiness* factor reflecting appositional growth.

The *length factor* can be assessed by different measurements.

In addition to the body height, in the

abruptly The values were recorded in 5 mm. Hg steps. Some correlation coefficients of blood-pressure are given in Appendix III, Table ~

PULSE RATE

The pulse rate was measured during 30 seconds immediately before and 30 seconds immediately after the injection of glucose and the mean of the two determinations was calculated.

The most important correlations between different somatic tendencies are given in Appendix III, T ble 1 which gives the following correlations in normal male population and relevant to the later discussion.

1 Body weight at 19 years of age is equally dependent on length, sturdiness, muscle and fat factors.

2. Body height at 19 years of age is not correlated with the fat factor

3. Weight and height at 19 years are closely correlated with weight and height at 13 years.

4 Birthweight is correlated with fat free weight t 19 years, but not with the fat-factor

5. Birthweight of child is correlated with the weight and height of the mother at 50 years but not with the weight or height of the father at this age

6. The fat factor of the male t 19 years is correlated with the weight of the mother at 50 years, while his height and fat-free weight are likewise correlated with the mother height but not with her weight.

The height and f t-free weight but not the fat factor of the male at 19 years of age are correlated with the f ther s weight and height t bout 50 years.

8 The parents heights, but not their weights, are correlated with one another at 50 years.

INTELLIGENCE TESTS

The intellectual endowments were assessed from the points scored by the probands in the intelligence test performed at induction. This test is based partly on the American Army general classification test (BITTNER, 194~) and modified in connection with the general intelligence testing of Swedish conscripts performed since 1944, and described by HUSÉN & HENRIKSSON (1951). The test has gradually been altered, so that the results from different years are not strictly comparable.

The test consists of sub-tests bearing on various aspects of intelligence. Thus, in 1959 the following types of subtests were used.

A. Questions illustrating capacity to understand instructions.
B. Vocabulary tests — *i.e.* differentiation of words with different meanings.
C. Spatial tests — assembly tests
D Questions illustrating ability of technical comprehension.

The results of the sub-tests are combined in mean score. To describe the subtests as well as the mean score, a 9-grade scale of a special form is used, the distribution of which is demonstrated in T ble 4 Correlations between different subtests and between subtests and the mean score are given in Table 5. In Table 6 means of mean scores from different years are demonstrated, from which it is evident that the results of the test in 1959 were noticeably low chiefly owing to a low mean point of sub-test A (Table 4).

The connection between intellectual level and somatic development, family structure and other sociologic conditions is demonstrated in Appendix III, Table 2. These figures coincide with the comparable

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The shoulder muscles, on which most interest was focused in the present investigation, seem to be particularly sensitive to testosterone (RUSSEL & WILHELMI, 1958)

LINDEGÅRD (1956) recognizes mainly two factors deciding muscularity i.e an environmental-stable factor closely related to the skeletal sturdiness factor and a labile factor dependent on training The range of the latter is believed to vary from muscle to muscle. The strength of the handgrip has been shown to be rather independent of muscular training and is believed to be less influenced by the labile factor Some correlation coefficients of the muscle factor are given in Appendix III, Table 5

Fat tissue The amount of fat tissue was assessed by two methods.

The *first method consisted of measurement of skin fold thickness by calipers for* assessing the amount of subcutaneous fat tissue (SKERLJ BROZEK & HUNT 1953) The values obtained by this method are *influenced by epidermal thickness and a* somewhat varying proportion between fat tissue and *connective tissue of the sub*cutis. Epidermal thickness may be regarded *as representing 2–3 mm* of the skin fold at the sites measured (EDWARDS, 1950)

The sites at which the measurements were *made in* the present investigation were *back* just caudal to the scapula, *lateral part of thorax* over the lower ribs, midway between the axilla and the iliac crest, and on the *abdomen* in the right midclavicular line at umbilical level. These measurements have been found to be closely correlated with the total amount of subcutaneous fat tissue (LINDEGÅRD 1956, PASCALE *et al.*, 1956)

The *second* method was based upon estimation of fat tissue *as judged by the* difference between the fat free weight and total bodyweight. Various methods have been used to assess the fat free weight. VON DÖBELN (1959) introduced a method based upon a regression calculation of values from measurements of various skeletal dimensions using the formula FFW = 15 1 (H F R) [?] where FFW is fat free weight, H the height in metres, F total femoral condylar breadth (left + right) and R total bistyloid radio-ulnar breadth (left + right) in centimetres.

The distribution and the amount of fat tissue is dependent not only on dietary habits, but also on endocrine factors. Before puberty the distribution of the fat is roughly the same for both sexes, while later the amount of fat appears to increase more in females (BROZEK *et al* 1953) As to the distribution in males, the fat tends to accumulate on the trunk, and, regarding actual age levels, particularly on its posterolateral aspect (EDWARDS, 1951)

According to BJURULF (1959) the fat factor is determined by two components, i e one constitutional, stable component represented by the number of fat cells and one labile dependent on environments represented by fat cell size. *The former* seems to be correlated with hair growth, especially on the chest. *Some correlation* coefficients of the fat factor are given in *Appendix III, Table 6.*

BLOOD-PRESSURE

Blood pressure was measured with a mercury Erkamanometer after the subject had been resting on the examination table for at least 8 minutes. The diastolic pressure was registered as the pressure at which the sounds *began to fall away*

bruptly The values were recorded in 5 mm. Hg steps. Some correlation coefficients of blood-pressure are given in Appendix III, Table 7

PULSE RATE

The pulse rate was measured during 30 seconds immediately before and 30 seconds immediately after the injection of glucose and the mean of the two deter minations was calculated.

The most important correlations between different somatic tendencies are given in Appendix III, Table 1, which gives the following correlations in a normal male population and relevant to the later discussion.

1 Body weight at 19 years of age is equally dependent on length, sturdiness, muscle and fat factors.

2. Body height t 19 years of ge is not correlated with the fat factor

3. Weight and height t 19 years are closely correlated with weight and height at 13 years.

4. Birthweight is correlated with fat free weight at 19 years, but not with the fat-factor

5. Birthweight of child is correlated with the weight and height of the mother at 50 years but not w th the weight or height of the father at this age.

6 The fat factor of the male at 19 years is correlated with the weight of the mother at 50 years, while his height and fat-free weight are likewise correlated with the mother s height but not with her weight.

The height and fat-free weight but not the fat factor of the male t 19 years of age are correlated with the father s weight and height t bout 50 years.

8 The parents heights, but not their weights, are correlated with one another at 50 years.

INTELLIGENCE TESTS

The intellectual endowments were assessed from the points scored by the probands in the intelligence test performed t induction. This test is based partly on the American Army general classification test (BITTNER, 1947) and modified in connection with the general intelligence testing of Swedish conscripts performed since 1944, and described by HUSÉN & HENRICSON (1951). The test has gradually been altered, so that the results from dif ferent years are not strictly comparable.

The test consists of sub-tests bearing on various aspects of intelligence. Thus, in 1959 the following types of subtests were used.

A. Questions illustrating capacity to under stand instructions.
B. Vocabulary tests — *i. e* differentiation of words with different meanings.
C. Spatial tests — assembly tests.
D Questions illustrating ability of technic al comprehension.

The results of the sub-tests are combined in mean score. To describe the subtests as well as the mean score, a 9-grade scale of a special form is used, the distribution of which is demonstrated in Table 4. Correlations between different subtests and between subtests and the mean score are given in Table 5. In Table 6 means of mean scores from different years are demonstrated, from which it is evident that the results of the test in 1959 were noticeably low chiefly owing to low mean point of sub-test A (Table 4).

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Blood-pressure was measured with a mercury Erkamanometer after the subject had been resting on the *examination* table for at least 3 minutes. The diastolic pressure was registered as the pressure at which the sounds began to fall away

Table 6. Mean scores of intelligence testing achieved in different years.

Born	Years of test	Series	n	mean ± SF	Mean of all tested 6th and 7th mil. reg. distr
1934	1952	A	22	4.8 ± 0.3	
1935	1953	A	14	5.6 ± 0.4	5.0
1936	1954	A	30	5.7 ± 0.3	5.1
1937	1955	A	20	5.4 ± 0.3	5.3
1938	1956	A	30	5.2 ± 0.3	5.2
1939	1957	A	28	5.1 ± 0.3	4.9
1940	1958	A	28	4.9 ± 0.3	5.3
1941	1959	A	20	4.6 ± 0.3	
1941	1959	B	485	4.6 ± 0.1	

according to: Instruktion för provledare 15. uppl. Militärpsykologiska Institutet, Lund 1959
In this table A means diabetics + their matched controls.
In 1951 induction-age was lowered from 19 to 18 years.

office clerks and youths attending school belong to this group.

2. Moderate muscular exertion, e. g. shop assistants, workers in light industry etc.
3. Considerable muscular exertion, e. g. farm labourers and workers in heavy industry etc.

b. *Mental requirements* of previous occupation

1. Employees in occupations requiring little mental work
2. Relatively independent work (this includes middle school and the first years of secondary school)
3. Intellectual work (professional people and foremen).

II *School-education*

1. Elementary school
2. Secondary schools. Folk high schools
3. Secondary schools (matriculated), Universities etc

III In the evaluation of *socio-economic level* the 3 social groups defined by the occupation of the father and generally recognized in Sweden were used.

Social group I. Owners and managers of large and middle-sized companies. Employees in responsible position — usually with university education.

Social group II. Owners of small businesses and employees in less responsible positions. This group also includes most owners of middle-sized farms.

Social group III. Workers, and men with small farmyards.

IV In the description of the *degree of urbanity* 4 classes were recognized.

1. Country
2. Small country towns (at least 500 inhabitants).
3. Towns with population of less than 100,000 inhabitants.
4. Towns with a population of more than 100 000 inhabitants.

QUESTIONNAIRES SENT TO MOTHERS OF PROBANDS

The questionnaires sent to the mother included questions on:

1. Incidence etc. of diabetes among relatives.

Table 4 Distribution of 465 persons in series B according to results of subtests and mean score of intelligence testing in 1959

test \ score	1	2	3	4	5	6	7	8	9
Subtest A	42	61	77	92	89	65	22	11	6
B	22	31	70	127	88	80	28	16	3
C	25	39	49	93	83	94	49	21	11
D	20	56	67	92	93	81	39	14	3
mean score	10	71	54	83	121	66	33	18	5

Table 5 Correlation coefficients between subtests, and subtests and mean score of intelligence tests

	A	B	C	D	mean score
Subtest A		0.69	0.51	0.57	0.82
B	0.69		0.51	0.59	0.82
C	0.51	0.51		0.56	0.78
D	0.57	0.59	0.56		0.81
mean score	0.82	0.82	0.78	0.81	

n = 465

For evaluation of significance see Appendix V

figures of HUSÉN (1951) Thus, there seems to be a correlation between test results and height, youths with higher intelligence being tall for age. This was however not accompanied by any increase of the length factor expressed as tibial or radial length.

No correlations were found with certainty between the test results and other somatic radicals, except for a readily understandable negative correlation between intelligence and bistyloid breadth, the latter being increased in those who had been doing heavy work.

A small number of children, especially in the families of the proband and his mother seemed to be associated with a higher intelligence level.

SOCIOLOGIC DESCRIPTION

The work and the social environments of the probands were described on the basis of the following factors with classes that could be graded in relation to one another

I *Nature of the work* was assessed by rough estimation of the demands placed on the physique and intelligence of the proband In each group 3 classes were recognized according to the following principles

a. *Physical requirements* of previous occupation

1 Little muscular exertion. Most

Table 6 Mean scores of intelligence testing achieved in different years.

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1940	1958	A	28	4.9 ± 0.3	5.3
1941	1959	A	20	4.6 ± 0.3	
1941	1959	B	465	4.5 ± 0.1	

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In this table A means diabetics + their matched controls.
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b. Mental requirements of previous occupation

1. Employees in occupations requir ing little mental work.
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In this table A means diabetics + their matched controls.
In 1934 induction-age was lowered from 19 to 18 years.

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QUESTIONNAIRES SENT TO MOTHERS OF PROBANDS

The questionnaires sent to the mothers included questions on:

1. Incidence etc. of diabetes among relatives.

2 [illegible]

2. Number of relatives of different types.
3. Ages of probands parents and grand parents.
4 Weights and heights of parents.
5 Birthweights of the proband and his sibs.
6 Mothers age at the menarche.
7 *Mothers age at first parturition.*

In series A 88.8 % of the mothers co-operated the corresponding figures for series Br and Bc being 90.3 % and 74 9 %

STATISTICAL METHODS

Arithmetical means (m) standard deviations (SD) standard errors of single determinations (SE) and coefficients of correlation (r) were calculated according to standard statistical methods.

The significance of a difference between two means was estimated with Students t test.

A difference was said to be almost significant ( ) significant ( ) and highly significant ( ) when the corresponding levels of probability were 0 05–0,01 0 01–0.001 and less than 0 001 respectively

Correlations built upon skewed distributions or when one variable contains only 3 or 4 classes were marked with □

Arithmetical means, standard deviations and distributions of all data of series B are given in Appendix II Table I

IV GENETIC-STATISTICAL ANALYSIS

GENERAL CONSIDERATIONS ON THE INHERITABILITY OF DIABETES MELLITUS

Familial factors are of importance in the causation of diabetes, as has been convincingly shown by the increased frequency of diabetes among relatives of diabetics (ALLAN, 1933, PINCUS & WHITE, 1933, GRUNNET 1957 STEINBERG, 1959 1961).

This increased frequency may be explained by genetic factors or by pseudohere dity the latter produced by environmental influences such as familial dietary habits. Strong evidence for the genetic alternative has been produced by investigations of twins (THEN BERG, 1939).

The nature of the inherited factor may be variable, nd the following possibilities deserve consideration

1. A uniform gene whose penetrance may be dependent upon environmental as well as constitutional factors. A possible alter native is then that of mild form of the disease in heterozygotes and more severe form in homozygotes.

– Various genes with similar or different patterns of transmission.

3. A common mutant gene whose penetrance is dependent mainly on hereditary factors.

I the evaluation of these alternatives the following considerations should be borne in mind.

a. If diabetes, independent of type of manifestation, is present in such proportions among the relatives of diabetics as may be expected according to one Mendelian type of inheritance it will argue for a uniform gene.

In the calculation of the gene frequency the fact that penetrance is lower in the lower ge classes must be taken into account. It does not seem probable that the frequency of manifestation ever reaches 100 % in an unselected group.

b. In view of the frequency of manifest diabetes a recessive inheritance by uni form gene must presuppose a fairly high frequency of the gene for diabetes.

One should then, after correction for age, observe frequency of diabetes among parents of diabetics, which though lower is of the same order as that among sibs of diabetics.

c. If diabetes is caused by different mutant genes with a recessiv manifestation, the frequency of each of the different genes for diabetes would be lower which would imply that the frequency of manifestation among the parents would decrease relatively more than among the sibs, for with an ex tremely low gene frequency the incidence of homozygosity among sibs will be 25 % as against 0 % for parents.

d. If diabetes is caused by a gene with dominant manifestation, the frequency of diabetic parents and sibs will be chiefly the same after correction for the increasing frequency of the disease with age

e. Also if the familial occurrence of dia

betes is due to a hereditary nature of factors favouring penetrance the considerations set forth above will be applicable.

In view of possibly hereditary influences on endocrine differences between the sexes, one might expect a certain degree of over morbidity among relatives of the same sex as the diabetics

In previous systematic investigations of the frequency of diabetes among relatives of diabetics it has proved difficult to correct the frequency of the disease for differences in age at onset in a random selection of diabetics. This difficulty has been avoided by some investigators by comparing the frequency of the combinations. diabetic + diabetic, diabetic + non-diabetic, and non-diabetic + non-diabetic among the parents of diabetics. Supposing a recessive pattern of inheritance and random mating, the frequency of these combinations should be p 2 qp q (the frequency of the diabetes gene is called p and that of the corresponding normal allele q) In these investigations the distributions obtained have been compatible with a competely recessive inheritance and a gene frequency of about 0.20 as pointed out by STEINBERG & WILDER (1952) (For survey of materials studied see STEINBERG, 1961) The figures in these investigations are consistent with a dominant inheritance only if the degree of penetrance is fairly low

PURPOSE OF ANALYSIS

The purpose of the analysis of the frequency of diabetes in different groups of relatives of the probands in series A and B was to find the best possible numerical expression for the risk of a relative of a diabetic being such a diabetes gene carrier as to be able to develop manifest diabetes.

PLAN OF INVESTIGATION

It appears possible, theoretically to calculate the incidence of genic aberrations with varying penetrance at different ages if the pattern of inheritance is known and data on the following points are available·

1 Penetrance at different age levels in the population studied.
2. Familial structure at different age levels.
3. Reliability of the information obtained.

In addition the following conditions must be fulfilled

4 *Random mating in the population.*
5 Marriage frequency and reproduction must be the same for different genotypes.
6. The phenotype must be distinguishable from other clinical pictures.

Under such conditions the expected frequency (L) in a group of proband families may be calculated in the following way· For each relative the expression P_x s r is calculated, where P_x is the penetrance at the age level x of the relative, s the reliability of data on the different groups of relatives and r the risk in a certain type of inheritance and gene-frequency in the population of being such a gene carrier as to be able to develop manifest disease. These values can then be added for all the relatives to a given group of probands, L being ΣP_x s r

1 PENETRANCE AT DIFFERENT AGE LEVELS

Since the age distribution of persons with manifest diabetes mellitus may have changed during the last decades, particularly since the introduction of insulin, and the increased expectation of life of the population, the age distribution must be based on recent examinations

The age distribution of the disease in the population forming the basis of the present investigation, was known from the investigation by SILVER (1958), which was founded on a search of the hospital records in Kristianstads län from the years

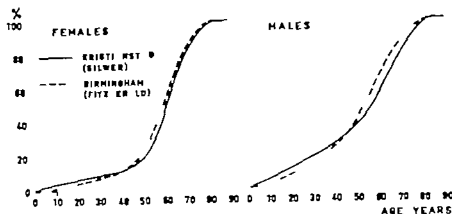


Fig. 1 Penetrance at different age levels, in per cent of maximal penetrance.

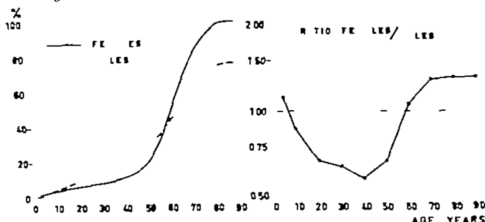


Fig. Penetrance of male and female diabetics at different age levels, in per cent of maximal penetrance among females.

1944–1953 and on written enquiries among all doctors practising in that area during the same time.

The curves for both sexes in Fig. 1 were constructed on the basis of cumulative percentages of diabetics in whom the disease became manifest within 10-year age classes – see Appendix II, Table 2. Only those cases were included in which the disease made its first appearance during the latest 10-year period, i.e. 1944–1953, so that the curves are largely independent of the overmortality of those in whom the disease occurs early in life. The general validity of the curves is seen on comparison with curves based on the distribution of newly discovered cases in Birmingham during the years 1949–1958 (FITZGERALD *et al.*, 1961). The slight difference between the curves may be due in part to the difference in the age distribution of the two populations.

The ratio between the risk of manifest diabetes occurring at different age-levels in males and females in SILWER's material is given in Fig. 2.

Table 7 Mean number of different types of relatives in series A, Br and Bc

Type of relative \ Series	A	Br	Bc
Sibs of probands	2.24	2.18	2.66
Sibs of fathers	4.79	4.35	4.76
Sibs of mothers	4.37	4.34	4.63
Paternal first cousins	8.67	9.17	8.11
Maternal first cousins	7.78	8.29	7.90

Table 8. Number of children of males and females of series Br who developed manifest diabetes before the ages of about 70 (grandparents) and about 50 (parents), respectively

Type of relative	paternal grand-father	paternal grand mother	maternal grand father	maternal grand-mother	grand-father	grand mother	father	mother
Number of relatives	24	43	19	44	43	87	26	1
Mean number of children	4.6	6.07	4.44	6.18	4.63	6.13	2.38	4.13

Ratio between number of children.
grandmother-grandfather 1.32:1 mother:father 1.74:1

Neither in series A nor in other series *on record* (STEINBERG & WILDER, 1952) did the occurrence of diabetes tend to vary with birth rank The ages of the sibs and *cousins are therefore normally distributed about the same means as those of the pro*bands — though the range of variation of *these two categories is somewhat larger* than that of the probands, but not to such an extent as to influence the degree of *penetrance — particularly since the curves* of both sexes in the region of 0—40 years may be regarded as linear

In order to obtain corresponding figures for the risk at the age levels of parents and grandparents, the duration of the observation period was noted, *i e* the present age or if the person had died, the age at death. At these age levels the curves can hardly be regarded as linear The groups of parents and grandparents were therefore divided into 4 equal parts according to the *duration of observation. Within every* fourth part the mean age was calculated and the corresponding degree of pene*trance was read from the curves in Fig 1 The mean of the 4 values found was re*garded as the degree of penetrance at the age level.

Thus, the expected penetrance, expressed in per cent of maximal penetrance was calculated for different types of male and female relatives of the probands in series A and B as well as of the probands in series B. The maximal penetrance — P_{max} — is a value, whose order must be assessed with due allowance for the various possibilities of inheritance and gene-frequency and is obtained as the quotient of *the number of diabetics found, divided by* the expected number of diabetic gene carriers capable of developing the disease

2. FAMILIAL STRUCTURE

The number of relatives of different types are given in Table 7

3. RELIABILITY OF INFORMATION

The reliability of the data given may be expected to vary with the type of relative and it is regarded as being best concerning sibs and parents, and less good concerning sibs of the parents and cousins. The reliability of the reports given by the probands in series B can be assessed by comparison between the frequency of diabetes among the probands and among the sibs and cousins of the probands. In the same way the incidence of diabetes among the parents and the sibs of the parents can be compared. In the latter group better knowledge of cases of severe diabetes requiring insulin can be expected compared with cases of mild diabetes not requiring insulin. In addition, knowledge of relatives who had the disease earlier in life may be expected to be better than that of those in whom the disease developed later i. recently. These assumptions were confirmed on examination of series B (Fig 3). As to the parent s sibs, then, half of those with insulin-treated diabetes were reported as against one eighth of those not treated with insulin. Knowledge was least regarding the parents sibs in whom the disease had appeared late and did not require insulin.

Supplementary information about the disease in the conscripts relatives was obtained from the mothers of the persons in series Bc, where the incidence of diabetes was not known by the 18 year old conscripts. Of the 179 mothers who cooperated, 9 reported total of 11 cases of diabetes among uncles, aunts and grand parents of the probands. This frequency

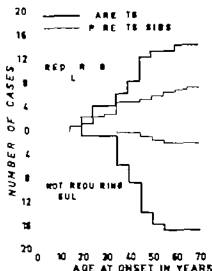


Fig 8 Ratio between diabetes requiring and not requiring insulin at different ag levels among parents and parents sibs, as reported by probands.

was supposed to hold for all of the conscripts who were registered that year and who had not reported any heredity for diabetes. In the calculation 228 persons in series Br 4 persons with manifest diabetes, and 179 persons in Bc whose mothers had given written answers, were subtracted from the total number of men registered, i.e 2659. The number of relatives of diabetics in the group of conscripts registered that year was thus about 340, i.e 13 per cent.

As far as series A is concerned, it may be assumed that the diabetic probands were more interested in the incidence of diabetes in the family and that they were therefore aware of most cases.

4. RANDOM MATING

Mating in the population studied was assumed to be random, at least concerning

the characteristics studied. This was supported to a certain extent by the fact that the geographical distribution of the birthplaces of the parents of the probands in series Br and those of *series A belonging* to the 6th registration district was largely the same as that of Bc.

5. REPRODUCTION OF DIFFERENT GENOTYPES

The calculation also requires that the reproduction and marriage frequency are the same for different genotypes

Some authors have claimed an increased fertility among diabetics (for survey see ARCINER & POST 1956) In the present *material, however the numbers of cousins* and of sibs of parents of relatives of diabetics did not differ appreciably from corresponding numbers in the normal population, *i e* series Bc. (Table 7)

Of particular interest in this respect was the agreement between the numbers of cousins, which may be regarded as an expression of the product of the factors marriage frequency and fertility

6. INHERITANCE OF DIABETES

As was described above extensive evidence is available for the significance of genetic factors in the occurrence of diabetes.

Many investigations suggest a uniform gene and, particularly autosomal recessive inheritance seems to fit in with the distributions and frequencies found The possibility of different types of diabetes with different modes of inheritance has, however been proposed by other research *workers, and* it cannot be assumed with certainty that all cases of diabetes mellitus is due to the same genic aberration

7 NUMERICAL EXPRESSIONS OF THE RISK OF BEING A CARRIER OF A GIVEN GENE

If random mating and identical reproduction and frequency of marriage of the *genotypes can be assumed, it is* possible to calculate with what probability different types of relatives of a given proband will be homozygous or heterozygous carriers of the proband s genes (HULTKRANTZ & DAHLBERG, 1927) The numerical expression of this probability was calculated for the diabetes gene for different gene frequencies in the population and assuming a dominant as well as a recessive mode of inheritance (See Appendix IV)

The investigation was based primarily on series A, which consisted of persons with early diabetes. If the frequency of diabetes among relatives of these persons is increased, the increase should be due to the same form of diabetes as in the probands.

The figures thereby obtained were checked in other materials. Differences between the values found and those expected were apparently due to diabetes with *an aberrant genetic* background.

In addition to the above mentioned procedures the frequency of the diabetes gene and the type of inheritance were estimated by investigation of the proportions non-diabetic + non-diabetic, non-diabetic + diabetic, diabetic + diabetic among parents of diabetics in series A

Further those members of series A and B were selected who had a diabetic parent whose mother or father was also diabetic The grandparents were divided into those requiring and those not requiring insulin. It was assumed that the latter group might contain an increased number of heterozygotes with mild diabetes or if different forms of diabetes inducing genes exist,

Table 9. Distribution of diabetic relatives in series A and B.

Type of relatives \ Series	Br	Dc	A
Brother	6		7
Sister	4		3
Father	26		8
Mother	17		2
Paternal uncle	19		10
Paternal aunt	15	1	6
Maternal uncle	11	2	6
Maternal aunt	12	1	3
Paternal grandfather	24	2	7
Paternal grandmother	44	2	7
Maternal grandfather	20	3	7
Maternal grandmother	48	1	8
First cousin	38		8

they would not be equally common in both groups.

In an attempt to assess the importance of exogenous factors, g dietary habits, the frequency of diabetes was studied among married partners of diabetics.

RESULTS AND DISCUSSION

The number of relatives with diabetes in series A and B are given in Table 9 Table 10 gives the expected frequency (L) of diabetics among different types of rela tives of probands in series A according to the formula L = ΣP described bove

Males and females were treated separately In series A the P_{max} was strikingly high for males as compared with that for females in view of the known situation in the population. It appeared possible that in the families of the male juvenile diabet ics there were genetically determined factors which particularly favoured the penetrance in males. The P_{max} for females was therefore considered to be more generally abl, for which reason, in the further calculations P_{max} for males was corrected for the ratio between P_{max} for males and females in Sjögren's material (Fig. 2)

A completely recessive mode of inheritance with a ratio of 0.15–0.25 between pathogenic and non-pathogenic alleles in the population studied seemed to best fit the observed values. A dominant inheritance with a gene ratio of the order of 0.05 and a degree of manifestation of about 20–30 % also largely agreed with the number of diabetics found. However the number of sibs expected would then be too small compared with that found.

The reliability of these calculations was checked in the following ways.

1 The number of diabetic probands and diabetic relatives of different types of probands in series B are given in T ble 11 where they are compared with expected alues for different assumed gene frequencies. The most useful values are those concerning the sibs, and the parents. As is clear from Fig. 3, knowledge of diabetes not requiring insulin in uncles and aunts was meagre so that it was thought advisable to correct the figures for the sibs

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Further those members of series A and B were selected who had a diabetic parent whose mother or father was also diabetic. The grandparents were divided into those requiring and those not requiring insulin It was assumed that the latter group might contain an increased number of heterozygotes with mild diabetes or if different forms of diabetes inducing genes exist,

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Mother	17		2
Paternal uncle	19		10
Paternal aunt	15	1	5
Maternal uncle	11	2	8
Maternal aunt	12	1	3
Paternal grandfather	21	2	7
Paternal grandmother	44	2	7
Maternal grandfather	20	2	7
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The reliability of these calculations was checked in the following ways.

1 The number of diabetic probands and diabetic relatives of different types of probands in series B are given in Table 11 where they are compared with expected values for different assumed gene frequencies. The most useful values are those concerning the sibs, and the parents. As is clear from Fig 3, knowledge of diabetes not requiring insulin in uncles and aunts was meagre so that it was thought advisable to correct the figures for the sibs

Table 10 Expected incidence of diabetes among relatives of 101 probands in series A assuming recessive inheritance, as well as dominant inheritance. (Series A consists of 119 probands, of whom 10 reported no heredity 6 were able to give information on only one of their parents and were therefore excluded, and 2 pairs of the probands were brothers: this left 101 for further analysis.)

Males						Recessive				Dominant	
				E	1/4	1/5	1/6	1/7	1/100	1/20	1/10
A	B	C	D	F	0.61	0.78	0 94	1 09	0.38	0.31	0.27
Brother	1 12	14	7		3.8	4 4	5 0	5 0	3 0	2.7	2.5
Father	1	41.5	8		6.4	6.5	6.5	6.5	7.8	6 9	6.2
Uncle	4.58	41.5	15		18.3	17 9	17.5	17.3	18.1	18.7	19 1
Grand father	2	80.3	14		13.5	15 1	14.8	14.5	15 2	15.8	16.2

Females											
				E	1/4	1/5	1/6	1/7	1/100	1/20	1/10
A	B	C	D	F	0 61	0. 8	0 93	1 11	0.36	0.30	0.28
Sister	1 12	7	2		1 9	2.2	2.6	2.9	1.5	1.3	1.2
Mother	1	20.3	2		3.1	3 2	3.3	3.2	3.8	3.4	3.0
Aunt	4.58	20.3	6		9 0	8.8	8 7	8.5	8 9	9 1	9 3
Grand mother	2	67 3	15		13.0	12 8	12.5	12 4	12.8	13.2	13.4

A Type of relative
B Number of certain types of relatives per proband.
C Penetrance at age level of type of relative given as percentage of maximal penetrance for each sex.
D Number of known diabetics among relatives of given type.
E Assumed diabetes gene frequency in population
F Pen trance among gene-carriers capable of developing diabetes on th assumption of different gene frequencies.

of the parents on the basis of the frequency of diabetes among parents. The data given by the probands in series Br on their grandparents were supplemented by information obtained from the mothers of the members of series Bc.

In the evaluation of the numbers of mothers and grandmothers with diabetes in series B correction must be made for the *increased number of children in females* with diabetes. Diabetic women at the 70-year age level have an increased number of grandchildren and consequently a greater possibility of being represented among grandparents of the 18 year old conscripts. This also holds for the middle-aged mothers with diabetes. The number of children of parents and paternal and maternal grandparents of series B with diabetes is given in Table 8.

2. 48.000 males were born in Scania 1934—1942. 139 subjects out of these were exempted from military service owing to diabetes. At 20 years the penetrance in males was on the average 10 % of P_{max}

On the basis of these figures the expected values given in Table 12 were calculated and compared with the observed number

3. In SILWER's material the incidence of

Table 11 Expected incidence of diabetes among 2,650 probands in series B and their relatives compared with observed values assuming recessive inheritance with a population gene frequency of 1/5—1/7 and dominant inheritance with population gene frequency of 1/100 and 1/20.

						Recessive			Dominant	
Males					F	1/5	1/6	1/7	1/100	1/20
					P	0.78	0.93	1.11	0.26	0.30
A	B	C	D	D	G	3.13 %	2.64 %	2.26 %	0.73 %	2.95 %
Proband	2650	8.6	6	6		8.0	6.7	5.8	1.9	7.8
Brother	3224	9.6	6	6		10.0	8.4	7.2	2.3	9.5
Father	2650	33.2	28	28		27.6	23.3	20.0	6.4	26.3
Uncle	11967	33.2	30	117		124.4	104.8	90.0	28.8	118.2
Grand-father	5318	80.4	44	96		100.8	84.9	72.7	23.3	95.6
Females										
Sister	3224	6	4	4		6.2	5.3	4.1	1.5	6.0
Mother	2650	19	17	14		16.2	13.7	10.6	3.8	15.7
Aunt	11967	19.5	28	82		73.0	61.8	47.6	18.9	70.7
Grand-mother	5318	70.0	82	120		116.5	95.3	78.9	27.0	112.8
		Total	231	448		482.4	407.0	333.9	111.9	462.4

A Type of relatives.
B Number of probands and different types of relatives.
C Degree of penetrance at age level of relatives and expressed as percentage of P_{max} (i.e. P_{max} women in series A).
D Number known by probands in series Br f diabetics among relatives of given type
D_1 = D corrected in following ways:
1) Mothers and grandmothers for effect of large number of children — see table 8.
2) Grandparents with the supplementary data obtained from mothers in series Be, see Table 9 assuming numbers of both sexes t be of equal size
3) Numbers of uncles and aunts are calculated according to the occurrence of diabetes among parents with the assumption that each parent had on the average 4.5 sibs.
I Assumed diabetes gene frequency in population.
F Penetrance among gene-carriers capable of developing diabetes on the assumption of different gene frequencies.
G Assumed maximal manifestation in per cent of population.

Table 12 Expected number of male diabetics born bet een 1934 and 1943 for different, assumed gene frequencies and modes of inheritance (Number of males born during this period in Scania = 48,000 Observed number of diabetics in military register = 138. Manifestation among males at 19 years = 10 % of P_{max})

	Recessive 1/5	Recessive 1/6	Recessive 1/7	Dominant 1/20
G	3.13	2.61	2.26	3.03
H	140.3	128.7	108.5	145.4

G. Assumed maximal manifestation in percent of population.
H Expected number of diabetics.

Table 13 Maximal frequency of diabetes (age level 70—79 years) found by SILWER (1954) in town and rural populations compared with calculated maximal frequency in per cent of total population for different assumed gene frequencies and modes if inheritance

	Morbidity age-level 0—79 (SILWER)		Recessive			Dominant
	rural	town	1/5	1/6	1/7	1/20
males	1 60	1.86	2.39	2.02	1 73	.31
females	2.08	2.83	3 13	2.64	2.26	3.03

diabetes was highest in the 70–79 year age class – see Table 13 The differences between the figures for rural and town population may be due to a higher frequency of diabetes inducing genes in the latter but is more likely due to an increased penetrance and more complete registration, especially of the elderly women and parti cularly those living in towns. The values may be compared with the expected mor bidity for recessive as well as dominant inheritance and different assumed frequencies of the diabetes gene.

4 91 of 101 probands in Series A had non-diabetic parents, while each of the remaining 10 had one diabetic parent Since the degree of penetrance at the age level of the parents was about *35 % for males and 20 % for females,* and assuming an autosomal recessive inheritance a probable proportion between the number of parental combinations of heterozygote + heterozygote· homozygote *+ heterozygote homozygote +* homozy gote seems to be 68 30· 3 Since this ratio also holds for (1 – p) 2 p (1 – p) p the frequency of the diabetes gene (p) may be assessed as about 0 18.

The figures are compatible with a dominant inheritance and P_{max} about 30 % if the gene frequency is assumed to be about *0 05*

5 The frequency of diabetic parents *with a diabetic mother or father was 3.6 %* for probands in series A and 3.7 % in series B

If the diabetic grandparents with known therapy in series B were divided into 60 *requiring* and *40 not requiring insulin,* the frequency of diabetes among children was 3.5 % and 3.8 %, respectively

The values obtained indicate after correction for age, that about 15 % of the children of diabetic parents will sooner or later develop diabetes and are well consistent with an autosomal recessive inheritance and a gene frequency of *0 15–0.20* The similar figures when the parents are requiring and not requiring insulin argue in some degree for a uniform diabetes gene and against the assumption that mild dia*betes not requiring insulin should be more* common in heterozygotes.

Here, too, a dominant inheritance with a gene frequency about 0 05 and P_{max} 30 % is compatible with the values found.

*As is apparent from the above compari*sons, good agreement was found between the expected and observed values on the assumption of an autosomal recessive mode of inheritance and then best with an assumed gene frequency of 0 1˜ – 0.20 and a maximum penetrance of *60–˜0 % in*

Table 14. Risk t birth of being homozygous diabetes gene carrier and risk at 20 years of ge of developing diabetes during rest of lif on the assumption of recessive inheritance and gene frequency of 0.20 in the population.

Type of diabetic relative( )	Risk at birth of being a diabetes homozygote.	Risk at 20 of developing diabetes during rest of life.	
		male	female
Sib	0.35	0.18	0.25
Parent	0.20	0.10	0.14
Child	0.20	0.10	0.14
Grandparent	0.12	0.06	0.08
Uncle or aunt	0.11	0.05	0.07
First cousin	0.08	0.03	0.04
Grandparents on same side	0.20	0.10	0.14
Grandparents on different sides	0.33	0.17	0.23
Parent + grandparent on side of other parent	0.58	0.30	0.40
Parent + sib of other parent	0.54	0.28	0.35
First cousins on different sides	0.15	0.05	0.06
Parent + first cousin on side of other parent	0.36	0.16	0.21

male homozygotes and of 80–90 % in female homozygotes. STEINBERG & WILDER (1952) found corresponding gene frequency of about 0.20.

A dominant inheritance with a gene frequency of about 0.05 and a maximum penetrance of about 25 % in males and of about 30 % in females could be an alternative but less probable interpretation of the observed values.

Assuming that the character is recessive, the values found would not argue against the assumption of a mild form of diabetes in few heterozygotes, and then particularly in females, though the frequency of penetrance in these heterozygotes would be low. Neither can the possibility of an aberrant form of diabetes chiefly in elderly persons be excluded.

The material does not lend itself well to evaluation of the last two possibilities.

If the character were recessive, one might imagine that the pseudoheredity due to such exogenic factors as dietary habits would probably be of less importance. The frequency of diabetes among married partners of the diabetics in series B — 8/172 among grandparents and 0/53 among parents, did not differ from that in the general population.

In the calculation of the risk of a given relative to develop diabetes, described in Table 14 and Fig. 4 the values given in Appendix IV are not absolutely accurate. It should also be taken into account that in the earlier generations there is a reduced risk of homozygosity among those who have attained certain age but have not developed diabetes.

Since it was not possible to find out the incidence of diabetes in generations before that of the grandparents, which was probably low these generations were treated as unknown.

As for the parents and grandparents,

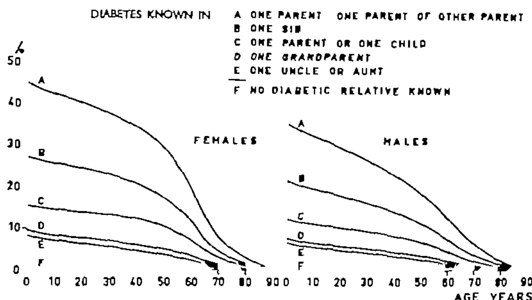


Fig 4. Risk of developing diabetes during rest of life, calculated for different age levels and different known diabetic relatives.

they were regarded as being 30 and 60 years, respectively older than the probands, the penetrance for the respective age levels being obtainable from Fig 1

No correction was made for non-diabetic sibs – in the present situation with relatively small numbers of sibs the values obtained are influenced only to a negligible extent by this factor

CONCLUSION

The assumption of a uniform autosomal completely recessive inheritance fits in best with the frequencies of diabetes observed among different types of relatives of probands in series A and B Assuming such an inheritance the risk of different types of relatives to develop diabetes at various age levels can be calculated.

V GROWTH AND DEVELOPMENT OF DIABETICS AND RELATIVES OF DIABETICS

PURPOSE OF INVESTIGATION

On the basis of clinical observations it has long been customary to recognize two types of diabetes, which have been called juvenile and adult diabetes according to age at onset. The former is said to occur preponderantly among individuals of slender body build, while the latter is described as being most common in association with overweight and as running more benign course (HOLSWORTH & KERR, 1939).

Different modes of inheritance have been suggested for the two types (CAMMIDGE, 1928 GRUNNET 1957). A point that has been widely discussed is whether diabetes is transmitted by uniform gene or by different genes, and secondly if a uniform gene is responsible whether the homozygote would suffer from more serious type of diabetes with an earlier onset than the heterozygote (HARRIS, 1950). The relatively higher incidence of adult diabetes in the families of juvenile diabetics argues for uniform gene. Thus, in the present investigation the number of members with adult diabetes in the families of juvenile diabetics would agree with the proportion expected, if the pathogenic gene is uniform

A question that then presents itself is whether juvenile diabetics in whom the disease has not yet become manifest are also of slender body build or whether the slenderness is evidence of the effect of the disease on some organs or systems particularly vulnerable during preadolescent and adolescent growth.

PLAN OF INVESTIGATION

In an attempt to elucidate these points measurements were made of the weights and heights of

a. diabetics at 13, about 18 and about 50 years of age,
b. males t 13 and 18 years in whom diabetes developed within the next few years,
c. varying types of relatives of approximately the same ages as in (a) of diabetics,
d. age-matched controls.

In the groups about 18 years old the relativ amounts of various types of tissue (skeletal, muscle and fat) were also assessed as well as the effect of diabetes heredity and the duration of manifest diabetes on these factors.

RESULTS AND DISCUSSION

The results are given in Tables 15–20 and in Fig 5.

In order to check whether the increased rate of growth found was not due to differences in composition between series Br and Bc, height and weight at 18 years were calculated for series Br also with the assumption of the same distribution of occupations as in Bc. It is clear from Appendix I, Table 2, that the difference found in height and weight at 18 years between B and Bc could not be ascribed to differences in occupation.

Neither could sociologic differences be-

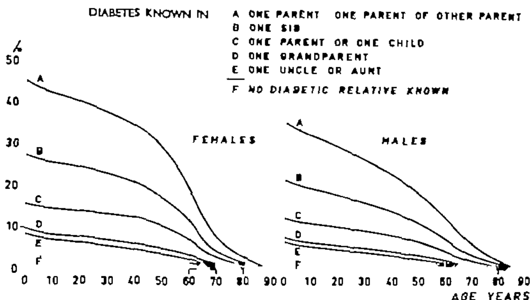


Fig 4 Risk of developing diabetes during rest of life, calculated for different age levels and different known diabetic relatives.

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CONCLUSION

The assumption of a uniform autosomal completely recessive inheritance fits in best with the frequencies of diabetes observed among different types of relatives of probands in series A and B Assuming such an inheritance, the risk of different types of relatives to develop diabetes at various age levels can be calculated.

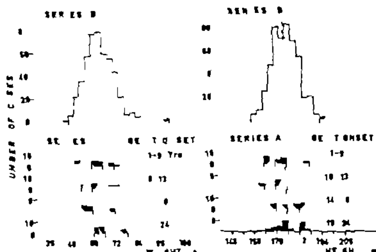


Fig. 5. Weight and height at induction in series A and B.

Table 16. Weight and height at induction.

		series		Weight mean (kg)	Weight SD	Height mean (cm)	Height SD
1		A	96	63.2	8.2	173.8	7.3
2		B	467	64.3	7.6	175.6	6.5
3		Br	237	64.5		176.3	
4		Bc	230	64.0		174.9	
	Diabetes known in						
5	Pat. grandparent	Br	64	63.9		175.5	
6	Mat. grandparent	Br	71	64.7		176.5	
7	Pat. uncle or aunt	Br	29	64.1		176.2	
8	Mat. uncle or aunt	Br	25	64.3		175.3	
9	Father	Br	26	65.0		176.4	
10	Mother	Br	17	61.2		177.9	
11	Sibs	Br	10	67.6		177.4	
	Age at onset						
12	1—9 yrs	A	24	58.8		169.2	
13	10—13	A	24	64.4		175.0	
14	14—18	A	24	62.4		176.1	
15	19—24	A	24	67.1		175.1	
16	matched controls	A	96	64.0		175.6	
	diff. 3—4			t=0.86		t=2.06	
	diff. 4—(9+10+11)			t=1.18		t=1.96	
	diff. 12—16			t=3.25		t=3.77	
	diff. 15—16			t=1.25			
	Correlation between age at onset and weight (height)			r=0.37		r=0.22*	
	Correlation between duration of diabetes and weight (height)			r=−0.37		r=−0.16	

From Nilsson

Table 15 Weight and height at 13 years of age.

		series	n	Weight mean (kg)	Weight SD	Height mean (cm)	Height SD
1		A	48	40.7	6.5	150.9	.8
2		B	114	43.7	6.8	155.1	8.1
3		Bc	60	43.6		154 7	
4	Matched controls, schoolregister	B	85	42.7		153.8	
	Diabetes known in						
5	Grandparent	Br	27	42.6		154 6	
6	Uncle or aunt	Br	17	43 1		154.2	
7	Parent or sib	Br	11	48.2		160.5	
	Age at Onset						
8	<13 years	A	20	38.3		147.3	
9	=13	A	8	41 4		153 0	
10	>13	A	20	42.8		153.6	
	diff. 1—2			t=2.65		t=3.11	
	diff. (3+4+5+6)—			t=2.46		t=2.51	
	diff 8—(9+10)			t=2.10		t=2.67	

tween Br and Bc explain the difference between weight and height of relatives and non-relatives at 50 years — c. f Appendix I Table 4

TENDENCIES IN PHYSICAL DEVELOPMENT OF RELATIVES OF DIABETICS

At the age of 18 (Table 16) and about 50 years (Table 20) the relatives of the diabetics tended to be taller and heavier than the controls. This tendency was most marked when a parent or sib was diabetic and was then discernible also in the 13-year old group (Table 15) This may be compared with WHITE's (1960) report on accelerated growth between the ages of six and sixteen of children of diabetic mothers. Other investigators for example HAGBARD OLOW & REINAND (1959) found no overgrowth when children of diabetic mothers were observed up to 16. At 18 years the skeletal sturdiness factor as well as the fat-free weight of children and sibs of diabetics was advanced for age. No sure tendency was found for relatives other than those referred to above.

The tendency to increased growth may be hereditary (GARN *et al.*, 1960) and possibly favour the penetrance of diabetes. If so, it may result in an over representation of overweight subjects among the relatives of diabetics.

Overweight was most marked at 50 years (Table 20), which may be ascribable to a stronger influence of body fat on weight. This overweight was greatest, at least in females, when there was paternal diabetes. It seems reasonable to assume that the penetrance of diabetes is dependent to a higher degree on hereditary growth-promoting factors in males than in females, *the risk of the disease developing in females also being increased by other factors, e g* reproduction

It is also believed that familial habits, e g over-eating, may result not only in overweight but also in a latent diabetes becoming manifest.

Table 18. Muscle and sturdiness factor and fat-free weight.

		series		Handgrip mean	Handgrip SD	Shoulder thrust mean	Shoulder thrust SD	Shoulder pull mean	Shoulder pull SD	Condylar breadth mean	Condylar breadth SD	Fat free weight mean	Fat free weight SD
1		A	107	41.3	7.3	53.3	11.3	37.2	7.6	9.54	0.48		
2		B	208	41.1	5.8	55.9	11.8	34.1	6.8	9.56	0.43	58.1	5.8
3		Bc	103	41.6		57.8		31.2		9.51		58.2	
	Diabetes known in												
4	Grandparent	Br	55	40.3		53.7		33.1		9.55		59.1	
5	Uncle or aunt	Br	30	41.2		55.7		36.4		9.61		59.3	
6	Parent or sib	Br	22	41.7		58.2		31.4		9.69		61.8	
	Age at onset												
7	1—9 yrs	A	30	38.4		43.7		34.3		9.31			
8	10—13	A	25	43.6		54.1		37.6		9.63			
9	14—18	A	25	44.3		56.0		35.6		9.00			
10	19—21	A	27	47.5		61.1		41.6		9.64			
	Correlation between muscle- and sturdiness factor and duration of diabetes				−0.10		−0.45		−0.22		−0.33		

Diff. 3—(4+5+6) $t = 1.48$ $0.2 > P > 0.1$
Diff. 3—6 $t = 2.74$ $0.05 > P > 0.01$

Table 17 Length factor

	series	n	Tibia mean (cm)	Tibia SD	Radius mean (cm)	Radius SD
	A	109	39.7	2.6	25.8	1.8
	B	208	39.0	2.8	25.6	1 1
	Bc	103	39 0		25.7	
Diabetes known in						
Pat. grandparent	Br	26	38.6		25.7	
Mat. grandparent	Br	29	39.2		25.8	
Pat. uncle or aunt	Br	18	38.4		25.7	
Mat. uncle or aunt	Br	12	39.5		25.8	
Father	Br	10	38.2		25.8	
Mother	Br	5	41 4		27.3	
Sib	Br	7	39 7		25 6	
Age at onset						
1— 9	A	30	38.6		24 9	
10—13	A	26	39.8		25.8	
14—18	A	26	40.2		26 0	
19—23	A	27	40.2		26.6	
Correlation between age at onset and length of tibia (radius)			r = 0.21		r = 0.34	
Correlation between duration and length of tibia (radius)			r = 0.21		r = 0.36	

TENDENCIES IN PHYSICAL DEVELOPMENT OF JUVENILE DIABETICS

There was a tendency to overweight of the 18 year old males who developed manifest diabetes within the following 5 years (Table 16). This tendency could not be accounted for by overheight. Owing to lack of age-matched controls, it was not possible to assess to what extent fat tissues, muscles or skeleton were responsible for this overweight, though some increase in the muscle and sturdiness-factors my be assumed.

The 13 year old group (Table 15) was too small to warrant comparison with controls.

Though most investigators have found overheight, overweight, and overmaturity of bones, in freshly diagnosed cases of juvenile diabetes (WHITE, 1960) this rule is not without exceptions (DANOWSKI, 1957).

Increase in weight without increase in height may be compared with the tendency to early sexual maturity as manifested by a lower age at menarche in girls who later on in life develop diabetes (WHITE, 1959). As shown by several investigators (for survey see TANNER, 1955) an early sexual maturity is preceded by a relatively high rate of increase of bodyweight, observable already during childhood. Until the age of 15 years this is accompanied by a corresponding increase in height, but not after the age of 18 years.

Some investigators (TANNER *et al* 1959) have found the urinary excretion of 17-ketosteroids to be increased in persons heavy for their height. This can be compared with the observation by WHITE (1959)

Table 20 Weight and height of parents (about 50 years old) of probands I series A and B.

		Series	Male N	Male weight mean (kg)	Male SD	Male height mean (cm)	Male SD	Female N	Female weight mean (kg)	Female SD	Female height mean (cm)	Female SD
1		A	91	77.7	10.3	173.8	5.3	96	70.0	10.5	164.9	6.0
2		B	345	78.8	9.6	175.0	6.2	376	68.9	14.7	161.2	5.3
3	N diabetic relativ	B	226	77.4		174.8		257	67.5		163.8	
	Diabetes known in											
4	Father	Br	23	81.1		175.8		20	72.8		165.2	
5	Mother	Br	38	80.4		175.3		43	68.8		164.7	
6	Sib	Br	29	77.3		173.3		23	69.0		161.3	
7	Themselves diabetic	Br	21	81.3		174.5		15	76.1		165.7	
8	duration ♂ 2—36 yrs ♀ 4—25	Br	11	80.2				8	76.2			
9	♂ 0— 7 yrs ♀ 0— 3	Br	10	82.3				7	76.0			
10	married partner of diabetic	Br	11	77.9		175.2		30	68.5		163.0	

diff 3—(4+5+6) t=1.88 (0.1>P>0.05) t=2.13 (0.05 >P>0.01)

Table 19 Fat factor

	series	n	Skinfold back mean	Skinfold back SD	*Skinfold lat. chest* mean	*Skinfold lat. chest* SD	*Skinfold abdomen* mean	*Skinfold abdomen* SD	*Weight minus fat free weight as per cent of weight* mean	*Weight minus fat free weight as per cent of weight* SD
	A	108	8.4	2.4	6.0	2.2	8.3	4.1		
	B	208	6.7	1.2	5.1	2.1	6.0	2.3	11.0	9.1
	Bc	103	6.0		5.1		5.8		12.0	
Diabetes known in										
Grandparent	Br	55	6.8		5.2		8.4		11.6	
Uncle or aunt	Br	30	6.7		5.1		6.1		11.1	
Parent or sib	Br	22	6.7		4.8		6.0		8.0	
Age at onset										
1—9	A	30	8.7		6.3		8.1			
10—13	A	25	8.3		6.0		8.7			
14—18	A	26	8.3		5.8		8.5			
19—24	A	27	8.2		5.9		7.8			
Corelation between fat factor and duration of *diabetes*			r = 0.12		r = 0.09		r = 0.01			

that the urinary excretion of 17 ketosteroids is increased at the onset of manifest diabetes, but seems to be reduced in the later course of the disease

On the other hand, in the juvenile diabetics *the values found for bodyweight, bodyheight, condylar breadth and muscle mass* at *about 18 years* were below those noted in the controls, the differences tending to be largest when the disease had become manifest before the age of 10–13 years. The amount of body fat seemed to be normal, or if anything, somewhat increased.

A tendency to underweight and under height was also noted among the 13-year old diabetics. In these, too, the degree of under-development *appeared to vary with the duration of the disease.*

The retardation of growth observed by several investigators (Wagner *et al* 1942, Engel, 1947 Bergqvist 1954 a, Leupold, 1960) may be compared with retardation of skeletal maturation in diabetics (Morrison & Bogan 1927) and late menarche in diabetic girls (Bergqvist 1954 c, Joslin *et al* 1959)

On the other hand, bodyweight of the *50-year-old diabetics in whom the disease* had not become *manifest* until after they had filled 20 years of age was, on the average, greater than normal, and also exceeded that of the relatives of diabetics referred to above.

A certain increase in growth prior to the appearance of diabetes may be attributable to increased endocrine activity *mainly with increased secretion of pituitary growth hormone* and possibly of testosterone. These hormones would then not only cause the increased growth, but also favour the penetrance of diabetes which in this investiga

Table 20 Weight and height of parents (about 50 years old) of probands I series A and B.

		Series	Male N	Male weight mean (kg)	Male SD	Male height mean (cm)	Male SD	Female N	Female weight mean (kg)	Female SD	Female height mean (cm)	Female SD
1		A	91	77.7	10.3	172.8	5.2	95	70.0	10.5	161.9	6.0
2		B	348	78.6	9.6	175.0	6.2	378	68.9	14.7	164.2	5.3
3	N diabetic relative	B	226	77.4		174.8		267	67.5		163.9	
	Diabetes know in											
4	Father	Br	23	81.1		175.8		20	73.8		165.3	
5	Mother	Br	38	80.4		175.2		42	68.8		161.7	
6	Sib	Br	28	77.2		175.3		23	69.9		161.3	
7	Themselves diabetic	Br	21	81.3		174.5		18	76.1		165.7	
8	duration ♂ 8—25 yrs ♀ 4—25	Br	11	80.3				8	76.3			
9	♂ 0— 7 yrs ♀ 0— 3	Br	10	82.3				7	76.0			
10	married partner of diabetic	Br	14	77.9		175.2		20	68.5		163.0	
	diff 3—(4+5+6)			t=1.68 (0.1>P>0.05)				t=2.13 (0.05 >1 >0.01)				

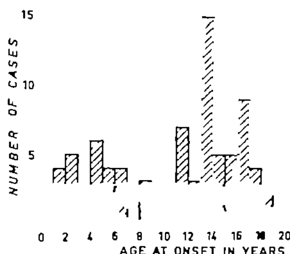


Fig 6 Age at onset in series A.

tion was highest at 13 years of age — see Fig 6 — an observation coinciding with that of most other workers (WHITE, 1959 CUTTLE & MACLEAN 1947) who have found a maximal manifestation at 11 in girls and at 13 in boys.

Since the capacity of growth hormone and testosterone to exert their effects is apparently to some extent dependent on an adequate production of insulin, (MILMAN et al., 1951 SCOW & CHERNICK, 1960 SIREK & BEST 1953) it seems reasonable that the retardation of growth of young juvenile diabetics is due to the successive reduction of endogenous insulin production some years after the onset of the disease and before the puberal growth spurt.

CONCLUSIONS

Judging from the observations set forth above,

a. growth of young male diabetics is retarded, particularly of those in whom the disease appears before puberty

b young males tend to be overweight before the disease becomes manifest,

c. factors responsible for this overweight assumedly also favour the manifestation of the disease, and

d. there appears to be no reason to assume any difference in the mode of inheritance between juvenile and adult diabetes on constitutional grounds.

VI. BIRTHWEIGHT OF YOUNG DIABETICS AND RELATIVES OF DIABETICS

PURPOSE OF INVESTIGATION

a. T study the birthweight of juvenile diabetics.
b. To study the birthweight of children of parents who later develop diabetes.
c. To study the birthweight when relatives other than the parents have diabetes.
d. T ascertain to what extent the over weight of children of women who later develop diabetes is due to the relatively large birthweight of children with a higher birth number
e. T ascertain to what extent the over weight of the infants is related to any overweight of the mothers who later develop diabetes.

PLAN OF INVESTIGATION

It has been observed in previous investigations (MOSENTHAL & BOLDUAN 1933, PYKE, 1956, FITZGERALD et al., 1961) as well as in the present study (See Table 8) that women who develop diabetes have, on the average, born more children than women in the rest of the population. Since birthweight has been found to increase with birth number (von WACHENFELDT, 1935, NAMBOODIRI & BALAKRISHNAN 1959) a higher birthweight of the children of prediabetic females may be ascribed partly to multiparity

I this investigation a correlation was found between birthweight and over weight of the mother (See Appendix III, Table 1) which can be compared with the similar observations of ODELL & MENCKERT (1945), GILBERT (1949), PIRART (1955).

As shown in Table 20 women who were diabetic at 50 years were overweight. In view of the general tendency of diabetic gene carriers to be overweight, a female overweight at 50 years may be related to an overweight during the childbearing years. It is clear from Table 21 however that the overweight of the middle-aged women also varied with the number of children they had born, c. f. PYKE & PLEASE (1957). It was therefore considered necessary to ascertain whether the increase in birthweight of infants born of mothers who later developed diabetes may be due to the overweight caused by multiparity or to overweight *per se*

The collection of data on series A and B was described in Chapter III. T elucidate the problems set forth above the following groups in these series were studied regarding birthweight and concerning the probands born at obstetrical clinics, also length at birth.

1 Children who developed diabetes before the age of 25 years.
2. Children who were sibs of juvenile diabetics.

Table 21 Correlation between number of children and weights of parents (Series B).

Number of children	1	2	3	4	5	6	[illegible]	8	9	10
Mother's mean weight (kg.)	65.2	66.1	67.2	66.8	71.3	6[illegible].1	73.3	[illegible]4.9	69.3	—
n	45	48	65	39	37	29	42	21	10	40
Father's mean weight (kg.)	79.5	[illegible]6.8	78.5	79.8	75.4	[illegible]4.8	78.5	72.1	[illegible]0.8	79.0
n	39	64	45	48	24	24	37	28	10	23

Correlation between mother's weight and number of children. $r = 0.17$
Correlation between father's weight and number of children. $r = 0.03$

3 Children of mothers who later developed diabetes.
4 *Children of fathers who later developed* diabetes.
5 Children with maternal heredity for diabetes.
6 Children with paternal heredity for diabetes.
7 *Children* without known diabetes in the family
8 Children with different numbers of sibs.
9 Children of non-diabetic parents of increased weight and height at 50

In series B the number of sibs was known, but not the birth rank of the probands or the weight of the mothers at the *time of delivery Therefore in order further* to elucidate the relationship between birthweight, and birth rank, and mother s weight, data were also collected on all children born at the Department of Obstetrics in Kristianstad in 1941 and at the Department of Obstetrics in Malmö 1960 which are hereinafter referred to as series K and M respectively *Further data were used* on infants born at the Department of Obstetrics in Lund between 1911 and 1930 (infants of primiparae between 1900 and 1922) (Lundh, 1925 v Wachenfeldt 1935). These infants will be referred to as series L. *Series L and K were collected from* areas of similar social structure as series B while series M was derived from a large *town Series M was partly chosen because* the weights of the mothers in the other series had generally not been noted in the records

The data on the probands in series A and B refer to boys only while the corresponding values in series K, L and M and for the sibs of the probands in A and B, were calculated *independently of* sex.

The differences between the weights of the boys and the girls in series K and M *and L were 109 g 90 g and 118 g* respectively The differences with sex correspond well with those found by other investigators (See Bakwin & Bakwin 1934 Engström & Falconer, 1960) In the calculations birthweights of less than 2000 g and corresponding lengths were not included in series K and M – these number represented 1 7 % and 1.8 % respectively The corresponding limit for series L was 2,500 g with the exception that none of the children of primiparae were excluded Neither were twins included, which represented *1 9 % (19/994) of the deliveries in* series K and 0.6 % (19/2978) in series M

Table 22. Birthweight and birthlength series A and B.

	Series			Weight			Length	
			n	mean (kg.)	SD	n	mean (cm.)	DS
	A		93	3.650	0.61			
	B		389	3.540	0.57	249	51.180	2.17
	Be	prob.	186	3.540				
	Be	sib	218	3.5 ●				
	A. matched controls		50	3.570				
Diabetes in Mother	Dr	prob.	12	3.850○		7	52.570○	
		sib	23	3.800●				
Father	Br	prob.	19	3.710○		13	51.540○	
		sib	34	3.700●				
Mat. relative	Dr	prob.	71	3.600○		43	50.950○	
		sib	96	3.720●				
Pat. relative	Dr	prob.	67	3.440○		38	50.880○	
		sib	92	3.510●				
Proband	A		93	3.650○				
Sib	A		135	3.660●				
>3 sibs	B		63	3.780○		40	51.950○	
Mat. wt>75 kg	B		49	3.740○		35	51.840○	
Mat. ht>170 cm.	B		22	3.840○		16	51.880○	
Pat. wt>85 kg	B		80	3.540○		31	51.390○	
Pat. ht>170 cm.	B		54	3.54		43	51.140○	

○ = only boys
● = boys and girls

RESULTS AND DISCUSSION

The birthweights in the above-mentioned groups of Series A and B are given in Table 22. The relation between the birth-weights of the children and the body weights of the mothers in series M and the birth rank in series K, L and M are summarized in Tables 23 and 24, respectively. The relation between birthweight and number of sibs in series B is given in Table 25 and the weights of the mothers in series B is related to their productivity in table 21.

Many authors have observed the birth-weight of children of prediabetic mothers to be increased (MILLER, 1945, MOSS & MULHOLLAND, 1951 JACKSON, 1952, PIRART 1955). This increase has been ascribed partly to constitutional factors which is supported by the observation that even when the father was a diabetic or prediabetic a certain increase in birthweight of

Table 23. Comparison between mother's weight and birthweight of child (series M).

Mother's weight \ Birth-rank	1			2			3—		
kg	n	n as % of total	mean (kg.)	n	n as % of total	mean (kg.)	n	n as % of total	mean (kg.)
45—49	6	0.5	3.08	3	0.3	2.82	4	0.7	2.72
50—54	40	3.3	2.74	30	3.5	2.97	19	3.3	3.10
55—59	129	10.5	3.12	83	9.5	3.16	49	8.5	3.17
60—64	249	20.3	3.26	187	21.7	3.35	105	18.3	3.40
65—69	283	23.0	3.39	168	19.5	3.51	101	17.6	3.38
70—74	232	18.9	3.46	149	17.3	3.63	110	19.1	3.56
75—79	138	11.2	3.57	128	14.9	3.62	83	14.4	3.72
80—84	70	5.7	3.53	55	6.4	3.69	48	8.3	3.45
85—89	40	3.3	3.64	32	3.7	3.71	22	3.8	3.78
90—94	25	2.0	3.78	13	1.5	4.00	12	2.1	3.68
95—	16	1.3	3.85	14	1.6	1.90	22	3.8	4.01
Total	1228			862			575		

Table 24. Birth weight and birth rank (Series L, K and M).

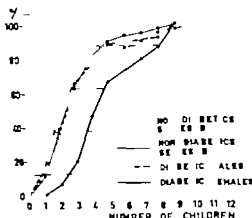
Birth rank	Series L		Series K				Series M		
	n	weight[1] (mean)	n	n as % of total	weight (mean)	length (mean)	n	n as % of total	weight (mean)
1	7000	3.35	475	47.8	3.44	50.4	1324	43.6	3.35
2	3577	3.59	258	26.0	3.58	50.9	969	32.1	3.47
3	2920	3.62	111	11.2	3.63	51.0	472	15.8	3.46
4	1774	3.68	60	6.0	3.73	51.5	169	5.6	3.46
5—6	1973	3.71	50	5.0	3.80	51.7	90	2.6	3.42
7—9	1215	3.75	28	2.8	3.71	51.2	9	0.3	1.66
10—	531	3.83	12	1.2	3.85	52.9			
Total	18990		994				3033		

[1] All firstborns included — at higher birth rank, birthweight <2.500 g and twins excluded.
[2] Birthweight <2000 g. and twins excluded

Table 25. Birthweight and number f sibs in Series B.

Number of sibs	n	weight mean
0	40	3.45
1	95	3.57
2	90	3.58
3	39	3.52
4	24	[illegible]
5—	33	3.54

Birthweight <2000 g and twins excluded



	Number of children		Corresponding birthweight	
	Diabetic	Non diabetic	Diabetic	Non diabetic
	Females		Females	
I	2.5	1.0	3.60	3.44
II	3.7	2.0	3.70	3.58
III	4.8	2.9	3.78	3.62
IV	7.1	6.5	3. 6	3.76
Mean	4.5	3.1	3.71	3.60

Fig. 7 Cumulative curv for parity of diabetic women, aged about 50, compared with normal curves.

the children has been demonstrable (JACKSON, 1952, SIEGLER & SIEGLER, 1960).

It appears that earlier investigators did not correct their results for multiparity and overweight of women who will develop diabetes.

After arrangement of the diabetic and non-diabetic mothers in series B into 4 equal groups according to the number of children they had born and construction of cumulative diagram (Fig 7), difference was found between the diabetic and non-diabetic women, in that the number of children born by the women, diabetic at 50, in each quarter was larger than the corresponding number for the non-diabetic group. For each quarter of the 2 groups the weights corresponding to the found number of children was calculated from Table 24 and the mean of the differences between these weights was taken as measure of the over weight ascribable to the larger number of children. The correction was made on the basis of values obtained from the analysis of series K, whose values roughly coincided with those of series L. Series M, which was derived from population of different social structure, showed a somewhat different pattern, for birthweight did not continue to increase after the second child.

It is clear from Fig. 7 that the birthweight of the child of the women who later develop diabetes should be corrected downwards by about 100 g owing to multiparity of the mother

On analysis of series M the infant's over weight at birth was found to be correlated, independently of birth rank with the weight of the mother at the time of delivery – See T ble 23. The mother s weight at the time of delivery may be regarded as proportional to that before pregnancy For it has been shown that the increase in weight during pregnancy is not related to the weight before pregnancy (IRGMAN, 1960). Neither does this increase in weight appear to be related with parity Since it was not known with certainty to what extent the overweight of the diabetic women was due to increase in weight before age t delivery or increase in weight between the age at which they had their children and 50 years, no correction could be made with certainty An increase of 5 kg. in the bodyweight of the mother as measured on admission, corresponded to an increase of

Table 23 Comparison between mother's weight and birthweight of child (series M).

Birth-rank / Mother's weight	1			2			3—		
kg	n	n as % of total	mean (kg.)	n	n as % of total	mean (kg)	n	n as % of total	mean (kg)
45—49	6	0.5	3.08	3	0.3	2.82	4	0.7	2.72
50—54	40	3.3	2. 4	30	3.5	2.97	19	3.3	3.10
55—59	129	10.5	3 12	83	9 5	3.16	49	8.5	3.17
60—64	249	20.3	3.26	187	21 7	3.35	105	18.3	3.40
65—69	283	23.0	3.39	168	19.5	3.51	101	17 6	3.38
70—74	232	18.9	3.45	149	17.3	3.63	110	19 1	3.56
75—79	138	11.2	3 57	128	14 9	3 62	83	14 4	3.72
80—84	70	5.7	3.53	55	6.4	3 69	48	8.3	3.45
85—89	40	3.3	3 64	32	3 7	3.71	22	3.8	3. 6
90—94	25	2.0	3.78	13	1 5	4.00	12	2.1	3.66
95—	16	1.3	3.85	14	1 6	1.90	22	3.8	4 01
Total	1228			862			575		

Table 24 Birth weight and birth rank (Series L, K and M).

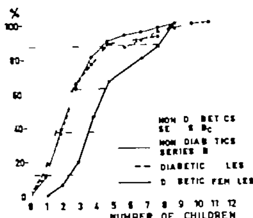
Birth rank	Series L		Series K				Series M		
	n	weight[1] (mean)	n	n as % of total	weight[2] (mean)	length[2] (mean)	n	n as % of total	weight (mean)
1	7000	3.35	475	47.8	3.44	50 4	1324	43.8	3.35
2	3577	3.59	258	26 0	3.58	50.9	969	32.1	3.47
3	2920	3 62	111	11.2	3 63	51 0	472	15.8	3.46
4	1774	3.68	60	6.0	3.73	51.5	169	5.6	3.48
5—6	1973	3.71	50	5.0	3.80	51 7	90	2.6	3.42
7—9	1215	3.75	28	2.8	3 71	51.2	9	0.3	3.66
10—	531	3 83	12	1 2	3.85	52.9			
Total	16990		994				3033		

All firstborns included — at higher birth rank, birthweight <2 500 g and twins excluded.
Birthweight < 2000 g. and twins excluded.

Table 25. Birthweight and number of sibs in Series B.

Number of sibs	n	weight mean
0	40	3.45
1	95	3.57
2	90	3.58
3	39	3.52
4	24	3.75
5—	33	3.84

Birthweight <2000 g. nd twins excluded



	Number of children		Corresponding birth eight	
	Diabetic	Non diabetic	Diabetic	Non diabetic
	Females		Females	
I	2.5	1.0	3.60	3.44
II	3.7	2.0	3.70	3.58
III	4.8	2.9	3.78	3.62
IV	7.1	6.5	3.76	[illegible]
Mean	4.5	3.1	3.71	3.60

Fig. 7 Cumulative curve for parity of diabetic women, aged about 50, compared with normal curves.

the children has been demonstrable (J cx son, 1932, Smoller & Smoller, 1960).

It appears that earlier investigators did not correct their results for multiparity and overweight of women who will develop diabetes.

After arrangement of the diabetic and non-diabetic mothers in series B into 4 equal groups according to the number of children they had born and construction of cumulative diagram (Fig. 7) difference was found between the diabetic and non-diabetic women, in that the number of children born by the women, diabetic t 50 in each quarter was larger than the corresponding number for the non-diabetic group. For each quarter of the 2 groups the weights corresponding to the found number of children was calculated from T ble 24 and the mean of the differences between these weights was taken as measure of the over weight ascribable to the larger number of children. The correction was made on the basis of values obtained from the analysis of series K, whose values roughly coincided with those of series L. Series M, which was derived from population of different social structure showed a somewhat different pattern, for birthweight did not continue to increase after the second child.

It is clear from Fig 7 that the birthweight of the child of the women who later develop diabetes should be corrected downwards by about 100 g owing to multiparity of the mother

On analysis of series M the infant s over weight t birth was found to be correlated, independently of birth rank with the weight of the mother at the time of delivery – See Table 23. The mother s weight at the time of delivery may be regarded as proportional to that before pregnancy For it has been shown that the increase in weight during pregnancy is not related to the weight before pregnancy (Ihrman 1960). Neither does this increase in weight appear to be related with parity Since it was not known with certainty to what extent the overweight of the diabetic women was due to increase in weight before ge at delivery or increase in weight between the age at which they had their children and 50 years, no correction could be made with certainty An increase of 5 kg. in the body-weight of the mother as measured on admission, corresponded to an increase of

Table 23. Comparison between mother's weight and birthweight of child (series M).

Mother's weight \ Birth rank	1			2			3—		
kg	n	n as % of total	mean (kg)	n	n as % of total	mean (kg.)	n	n as % of total	mean (kg)
45—49	6	0.5	3.08	3	0.3	2.82	4	0.7	2.72
50—54	40	3.3	2.74	30	3.5	2.97	19	3.3	3.10
55—59	129	10 5	3.12	83	9.5	3.16	49	8.5	3.17
60—64	249	20.3	3.28	187	21 7	3.35	105	18.3	3.40
65—69	283	23.0	3.39	168	19.5	3.51	101	17 6	3.55
70—74	232	18.9	3 45	149	17.3	3.63	110	19 1	3.56
75—79	138	11 2	3.57	128	14 9	3.62	83	14 4	3.72
80—84	70	5.7	3.53	55	6 4	3 69	48	8.3	3 45
85—89	40	3.3	3 64	32	3.7	3.71	22	3.8	3.76
90—94	25	2.0	3.78	13	1.5	4.00	12	2.1	3 64
95—	16	1.3	3.85	14	1 6	3.90	22	3.8	4 01
Total	1228			862			575		

Table 24 Birth weight and birth rank (Series L, K and M).

Birth rank	Series L		Series K				Series M		
	n	weight[1] (mean)	n	n as % of total	weight[1] (mean)	length[1] (mean)	n	n as % of total	weight[1] (mean)
1	7000	3.35	475	47 8	3.44	50.4	1324	43.8	3.35
2	3577	3.59	258	26.0	3.58	50.9	969	32.1	3.47
3	2920	3.62	111	11.2	3.63	51 0	472	15.8	3.46
4	1774	3.68	60	6.0	3 73	51.5	169	5.6	3.48
5—6	1973	3.71	50	5 0	3.80	51 7	90	2.6	3.42
7—9	1215	3 75	28	2.8	3 71	51.2	9	0.3	3.66
10—	531	3 63	12	1.2	3.85	52.9			
Total	18990		991				3033		

All firstborns included — at higher birth rank, birthweight < 2.500 g and twins excluded.
[1] Birthweight <2000 g. and twins excluded

Table 25 Birthweight and number of sibs in Series B

Number of sibs	n	weight mean
0	40	3.45
1	95	3.57
2	90	3.53
3	39	3.52
4	24	3.75
5—	33	3 54

Birthweight <2000 g. nd twin excluded

VII. INTELLECTUAL ENDOWMENTS OF YOUNG DIABETICS AND YOUNG MALE RELATIVES OF DIABETICS

The intellectual endowments of diabetic children has received wide attention. Some authors have found no differences between diabetics and controls, others have found diabetics to be over-intelligent or under intelligent for ge (For survey see DA KOWSKI, 1957).

Below comparison is given of the scores achieved by diabetics, non-diabetic relatives of diabetics, and non-diabetic controls at tests performed at induction (Table 26).

The intelligence level of the 18 year old males who had diabetes mellitus or developed the disease within the following 6 years was found to be higher though not significantly than of ge-matched controls.

No difference was found between the relatives of diabetics and the controls. The differences obtained between different types of relatives is probably due to a correlation between the intelligence of the proband and his knowledge of the disease in other members of the family

The intellectual level did not seem to vary with the duration of the disease.

Series A and B are not strictly comparable because of modifications of the tests during the years in question, and especially because the level of the results of the test were noticeably low in 1959 (See Table 6).

The value of the tests should not be over estimated. The difference found might have been due to an increased degree of

Table 26. Results of intelligence tests (mean score).

		mean	SD
Series A	96	5.21	1.96
Age at onset of diabetes (yrs)			
1—9	21	5.38	
10—13	21	5.00	
14—18	24	5.67	
19—23	24	5.04	
Matched controls	96	4.73	
Series B	465	4.49	1.78
Diabetes known in:			
Grandparent, uncle or aunt	165	5.03	
Parent	44	4.34	
Sib	19	4.21	
Non-relatives	250	4.28	

Diff. Series A and its matched controls: t = 1.82, 0.10 > P > 0.05

about 100 g in the weight of the child, independently of birth rank (Table 23)

One might imagine that both the parity and the weight of the mother are dependent on the age of the mother and that the dif ferences observed are due to increased birthweight ascribable to the higher age of the mother In series L (Lunde, 1925, Wachenfeldt 1935) no correlation was found between the age of the mother and the birthweight of the child. Nor was any such correlation found in the other series.

As a cause of overweight common to the mother and the child at birth the state of nutrition of the mother may be considered and is believed to influence birthweight to some degree (for survey see Abolins, 1961) This factor cannot be assessed in the pre sent investigation, but it was probably of less importance in this material emanating from a generally well nourished population.

Many investigators, Bakwin & Bakwin (1934) for example, have found a higher birthweight when the parents belong to a higher socio-economic level. This may be related to nutrition but seems to be inde-pendent of other factors referred to above Some sociologic differences exist between diabetics and the normal population, as was discussed in Chapter II and is demon-strated in Appendix I. The material, how ever was too small for an analysis of this influence upon birthweight, which was considered of less importance in the pre-sent investigation.

One might suspect that the overweight observed when the father is a future dia betic is only a consequence of the correla tion between the weights and heights of the parents (Appendix III, Table I) It is clear from Table 20 however that the weights and heights of the wives of diabetics in the present material did not deviate from normal.

The 18 year old males who developed diabetes before the age of 25 showed an increased skeletal and muscular develop-ment for age at 18 years (Chapter V) It is clear from Appendix III Table I that birth-weight was related to skeletal and muscle factors in 18 year old males.

The overweight at birth was, however not directly proportional to the increased risk of development of diabetes owing to increased rate of growth. For the birth-weights of the juvenile diabetics were the same as those of their normal sibs. Children with a low birthweight tended to develop the disease at the same time as those with a high birthweight — as a matter of fact there was, if anything, a tendency of those with a lower birthweight to develop the disease earlier ($r = 0.20$)

Length at birth generally varied with birthweight, so that the overweight of prediabetic parents as well as of children with a high birth number was probably due to a general increase in growth and not only to an isolated change of e g fat or fluid.

No overweight or underweight was found among probands when more distant rela tives were diabetic.

CONCLUSION

A child has a tendency to be overweight at birth if the mother develops diabetes later in life This overweight is, however due partly to the larger number of children born by the prediabetic women, since the overweight of the child at birth appears to be related to its birth number Further the weight of the child is possibly corre-lated with the overweight of the prediabetic mother the maternal overweight appearing to influence the birthweight of the child.

If the father or sibs are diabetics, the overweight at birth will be less marked and will be of the same order as that for persons who will later develop diabetes.

VII. INTELLECTUAL ENDOWMENTS OF YOUNG DIABETICS AND YOUNG MALE RELATIVES OF DIABETICS

The intellectual endowments of diabetic children has received wide attention. Some authors have found no differences between diabetics and controls, others have found diabetics to be over-intelligent or under intelligent for age (For survey see D kowski, 1957).

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The intelligence level of the 18 year old males who had diabetes mellitus or developed the disease within the following 6 years was found to be higher though not significantly than of age-matched controls.

No difference was found between the relatives of diabetics and the controls. The differences obtained between different types of relatives is probably due to a correlation between the intelligence of the proband and his knowledge of the disease in other members of the family.

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Sib	19	4.21	
Non-relatives	250	4.26	

Diff: Series A and its matched controls: $t = 1.82$, $0.10 > P > 0.05$

penetrance and an earlier manifestation of the disease in larger towns and in certain sociologic group (c. f Chapter II) While the population of Malmö represents 25 % of that of Scania, the diabetics living in Malmö represent 30 % of series A. As is apparent from Table 7 the degree of urbanity as well as socio-economic level is correlated with the results of the intelligence tests

VIII INTRAVENOUS GLUCOSE TOLERANCE TEST

The purposes of the investigation were:

A. To appraise the value of the intravenous glucose tolerance test with rapid injection of glucose as a measure of the risk of young males developing diabetes mellitus, as well as to appraise the effect of priming with adrenal glucocorticoids on the shape of the glucose curve

B. To assess the effect of total body volume and the ratio between different tissues (skeletal, muscle and fat) on the shape of the glucose curve

COMMENTS ON METHODS

REMARKS ON INTERPRETATION OF GLUCOSE TOLERANCE TEST

In the beginning of the investigation the rate at which the blood sugar fell within the volume in which the glucose was initially distributed was taken as a basis for evaluating the risk of the development of diabetes, as well as for differentiating different tissues as to their influence upon the glucose curve. The effect of other factors on this fall must therefore be defined as accurately as possible

Some factors which independently of the type of tolerance test might disturb the course were assumed to be-

a. Physical fitness.
b. Physical exertion just before test.
c. Infections and other forms of exogenous stress.
d. Psychic stress.
e. Dietary habits, particularly carbohydrate intake the last few days before the examination.
f. Diurnal rhythm.

a. *Physical fitness.* The degree of physical fitness of the young males varied — they had all been in the armed forces 1–60 days before the glucose tolerance test was performed. Also heaviness of the work in the previous occupations of the recruits varied. Many of them had come straight from school, where they had been preparing for their final examination and had had no time for physical exercise. Since the males were all about 19 years old, and apparently healthy the possibility of decreased glucose tolerance because of prolonged physical inactivity (BLOTNER, 1945) can be ignored.

The state of physical fitness might have improved with the number of days the men had been in the armed forces, for which reason the blood sugar level was studied for any correlation with the number of days that had elapsed since they had been enlisted. It was found that the fall in the blood sugar level tended to be more rapid with the duration of military service (see Table 27), though allowance must be made for a certain unevenness of the distribution of the material according to physical fitness. The glucose tolerance curve was not found to vary with the type

penetrance and an earlier manifestation of the disease in larger towns and in certain sociologic group (c.f Chapter II) While the population of Malmö represents 25 % of that of Scania, the diabetics living in Malmö represent 30 % of series A. As is apparent from Table 7 the degree of urbanity as well as socio-economic level is correlated with the results of the intelligence tests.

demonstrable between pulse rate and the results of the glucose tolerance test.

e Dietary habits, especially carbohydrate intake at the time of the examination. – On the days before the examination the men received their ordinary ration, which was considered to contain sufficient carbohydrate to exclude the possibility of the glucose tolerance test being influenced by carbohydrate deficiency (Malmros, 1928, Conn, 1940, Wilkerson *et al* 1950). The possibility of a high carbohydrate diet resulting in increased tolerance has been suggested by some authors (Sweeney 1927) but denied by others (Goldberg & Luft 1948, Tunbridge & Allibone, 1940) – this possible source of error was ignored. The men were instructed not to eat during the last 12 hours before the examination. They were also requested not to smoke during this period, it having been shown by Lundberg & Thyselius-Lundberg (1931) that smoking tends to raise the blood sugar

f *Diurnal rhythm.* There is probably diurnal rhythm of the blood sugar level and especially of the tolerance for exogenous glucose. This rhythm may be due to the *assimilation and dissimilation phases* of the liver (Forsgren, 1935). The tolerance tests were performed between 7 30 and 10.30 a. m. The results of the glucose tolerance test were not found to vary with time within these limits.

COMPARISON BETWEEN ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TESTS

Circumstances allowed the choice between an oral tolerance test and an intravenous test with rapid injection of glucose, but not an intravenous test with slow infusion of glucose.

Each of the two methods available have

certain advantages and disadvantages. They probably differ in the following respects:

g. Duration of uptake of glucose.
h. Hepatic uptake and outflow of glucose.
i. Renal excretion of glucose.
j. Secretion of insulin and consequent increase *of factors raising* the blood sugar level.

g *Duration of uptake of glucose* – The absorption of glucose on oral administration varies and seems t be modified, among other things, by the rate of gastric emptying (Watson, 1937 Everson 194.), intestinal peristalsis (Cummins & Almy 1953) dilution of glucose in the intestines (Cummins & Jussila, 1956, Annegers, 1961), and the capacity of the active bsorption process (Crane & Krane, 1956, Crane, 1960). These factors, which are difficult to assess, are completely avoided by intravenous injection of the glucose.

A disadvantage of the oral test, as far as the present investigation is concerned is also the fact observed by Duncan *et al.* (1958) that the peak of the tolerance curve is probably reached quicker in persons of heavy bodybuild, while obesity *per se* seems to be combined with a retardation of the absorption of glucose.

h. *Hepatic uptake and outflow of glucose* According to most authors (Somers & Levine, 1952, Searle & Chaikoff 1952), the outflow of glucose from the liver falls to low alues if the blood sugar level is elevated, and returns to normal gain with the falling blood sugar level. In manifest nd latent diabetes the hyperglycaemia does not seem to be accompanied by a normal fall of the hepatic glucose outflow (Somers & Levine, 1952, Isselutz *et al* 1960). It seems reasonable to assume a difference in the reaction of the liver to

Table 27 Correlation coefficients between results of glucose tolerance test and
A. factors possibly stimulating increase of muscle volume and strength
B pulse rate

		Blsr[illegible] $n = 203$	Blsr[illegible] $n = 202$	Total index $n = 202$
A	Number of days after enlistment	—0.22	—0 15	0 01
	Manual labour in earlier occupation	—0.00□	—0.01□	—0.05□
B	Pulse rate	—0.00	—0 07	0.08

For evaluation of significance see Appendix \

of work the subjects had been doing, *i e* heavy manual labour or light work (Table 27)

b *Physical exertion just before test* Physical exertion is believed to increase the rate of uptake of glucose even in the absence of insulin – probably by formation of a humoral factor (GOLDSTEIN *et al.*, 1953, GOLDSTEIN 1961) and a relative "anaerobiosis (RANDLE & SMITH, 1958) Thus, following severe physical exertion a lower or flatter glucose tolerance curve has been obtained (STRANDELL, 1934)

In the present investigation most of the subjects were examined in the bed in which they had been lying during the night. 54 of them had, however come to the examination room the same morning, but they had avoided all unnecessary physical exertion that morning and they had rested for at least 2 hours before the examination. No differences were found between the mean values of this group and that of the remainder

c. *Infections and other forms of exogenous stress* Before the examination the subjects were examined and questioned whether they had had any recent infections which has been shown to rise the blood sugar level (BERG, 1926, LAWRENCE & BUCKLEY 1927 TUNBRIDGE & ALLIBONE, 1940) and if they had, the examination was postponed. All together 3 had been on the sick-list the week before the examination because of infections of the respiratory tract, but they had returned to duty before the time of the examination – the previous infections do not appear to have influenced the blood sugar level

Recruits are usually vaccinated against smallpox the first week after they have enlisted – in 2 of the men the glucose tolerance test was postponed because of marked reaction to the vaccine.

d. *Psychic stress.* The first few weeks of military service often imply a certain psychic stress, which is believed to raise the blood sugar (CANNON 1947) In addition, venous puncture for the test undoubtedly implies a psychic strain for many probably causing an increase in the pulse rate, which was therefore measured both immediately before and after the glucose tolerance test. It is, however clear from Table 27 that no signs of a correlation were

demonstrable between pulse rate and the results of the glucose tolerance test.

Dietary habits, especially carbohydrate intake at the time of the examination. – On the days before the examination the men received their ordinary ration, which was considered to contain sufficient carbohydrate to exclude the possibility of the glucose tolerance test being influenced by carbohydrate deficiency (MALMROS, 1928, CONN, 1940, WILKERSON *et al.* 1960). The possibility of a high carbohydrate diet resulting in increased tolerance has been suggested by some authors (SWEENEY 1927) but denied by others (GOLDBERG & LUFT 1948, TUNBRIDGE & ALLIBONE, 1940) – this possible source of error was ignored. *The men were instructed not to eat during* the last 12 hours before the examination. They were also requested not to smok during this period, it having been shown by LUNDBERG & THYSELIUS-LUNDBERG (1931) that smoking tends to raise the blood sugar

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Each of the tw methods available have certain advantages and disadvantages. They probably *differ in the following respects:*

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j. Secretion of insulin and consequent increase of factors raising the blood sugar level.

g. *Duration of uptake of glucose.* – The absorption of glucose on oral administration varies and seems to be modified, among other things, by the rate of gastric emptying (WATSON 1937, EVENSON, 1942), intestinal peristalsis (CUMMINS & ALMY 1953), dilution of glucose in the intestines (CUMMINS & JUSSILA, 1956 ANNEGERS, 1961), and the capacity of the active absorption process (CRANE & KRANE, 1956, CRANE, 1960). These factors, which are difficult to assess, are completely avoided by intravenous injection of the glucose.

A disadvantage of the oral test, as far as the present investigation is concerned is also the fact observed by DIETZE *et al.* (1958) that the peak of the tolerance curve is probably reached quicker in persons of heavy bodybuild, while obesity *per se* seems to be combined with retardation of the absorption of glucose.

h. *Hepatic uptake and outflow of glucose.* According to most authors (SOSKIN & LEVINE, 1952, SEARLE & CHAIKOFF 1952), the outflow of glucose from the liver falls to low values if the blood sugar level is elevated, and returns to normal again with the falling blood sugar level. In manifest and latent diabetes the hyperglycaemia does not seem to be accompanied by normal fall of the hepatic glucose outflow (SOSKIN & LEVINE, 1952, ISSEKUTZ *et al.* 1960). It seems reasonable to assume difference in the reaction of the liver to

glucose given by the intravenous route and the oral route, respectively owing to a special sensitivity to the concentration of the glucose in the blood in the portal region (SCOTT & CORNFIELD, 1954)

i *Renal excretion of glucose* The loss of glucose in the urine following intra venous injection of 25 g. glucose is nor mally insignificant because the blood sugar level falls rapidly below the renal thres hold for glucose. Several investigators have found the amount of glucose excreted in the urine after injection of 25 g glucose to be less than 2 g (AMATUZIO *et al.* 1953 IKKOS & LUFT 1957)

Neither will the amount of glucose lost with the urine generally affect the results *of the test on oral administration of glu*cose. However inter-individual differences *in the renal threshold must be considered,* which are probably of greater importance *in the oral test* where the blood *sugar* level is near the threshold level for a longer period.

j *Secretion of insulin and consequent increase of factors raising the blood sugar level* The interval between the rise in blood *sugar concentration* and increased insulin activity is not known with certainty *Evidence available has made is probable* that the insulin promptly adjusts itself to *the glucose level (ANDERSON et al 1956* METZ, 1960) It seems reasonable to expect *an increased activity after a few minutes* but a maximal effect not before 20—30 mi*nutes after the initiation of hyperglycaemia.* In diabetes the secretion of insulin is re tarded (*YALOW & BERSON* 1960)

It is not properly understood how early *increased insulin activity* and consequent fall of the blood sugar level initiates *homeostatic processes.*

The oral test, in which the glucose is *taken up by the body for a longer period* and the rise in the blood sugar persists for a longer time than on rapid *intravenous* injection of glucose, might be more sensitive to the *effect of increased insulin se*cretion and other endocrine factors which do not exert their full *effect in association* with the very transient increase in the blood *sugar following intravenous ad* ministration.

On the basis of these comparisons it appears likely that the more physiological oral test might have advantages regarding the demonstration of potential diabetics but that the rapid intravenous test, owing to the absence of factors influencing absorption and difficult to assess, is superior for describing the effect of non-endocrine factors, and particularly of constitutional influences, on the results of the tolerance test

APPRAISAL OF INTRAVENOUS GLUCOSE TOLERANCE TEST

In the *present* investigation the intra venous tolerance test was chosen with the injection of 100 cc. of 25 % glucose This technique has in recent years been widely used in routine clinical work and in experimental studies (HAMILTON & STEIN *1942, GREVILLE, 1943,* AMATUZIO *et al* 1953, DUNCAN 1956 a, IKKOS & LUFT 1957 WEST & WOOD, 1959) The results are described by *inter alia* the rate at which the blood sugar falls after the rapid initial rise In order to determine this rate *independently* of the glucose level, the blood sugar values are plotted on semilogarithmic paper and then show a curve which is roughly linear and whose slope is taken as an expression of the rate of the glucose uptake by the tissues. This rate (k) can be expressed *according to the* general formula for a monomolecular reaction

$$k = \frac{2.303 (\log C_1 - \log C_2)}{t_2 - t_1}$$

where C_1 and C_2 designate the concentration of the glucose at two consecutive times t_1 and t_2 during the period the blood sugar is falling from maximum to fasting level.

It is still debatable whether the glucose concentration used for the calculation should be the concentration of the total glucose (HAMILTON & STEIN 1942, CONARD, 1955, IKKOS & LUFT, 1957, WEST & WOOD, 1959) or the concentration of the glucose above the fasting blood sugar level (AMATUZIO et al. 1953, DUNCAN 1956 b) – in the former case k is hereinafter referred to as total index (T. I.) and in the latter as increment index (I. I.).

In an attempt to assess the usefulness of T. I. compared with I. I. a preparatory examination was made on a total of 50 in-patients of Departments of Internal Medicine and Obstetrics. The patients selected for this test had occasionally had glycosuria or had born children with a birthweight of more than 4 kg. Their ages ranged between 18 and 56 years (mean 33 years). The glucose in the capillary blood was measured according to Hagedorn-Jensen's method immediately before and 5, 10, 20, 45 and 60 minutes after injection of 25 g. of glucose intravenously. Zero time was said to be the moment the injection was begun.

The material was divided into 4 equally large groups according to the blood sugar value measured 5 minutes after the injection, which was considered the best interval for determining the maximal blood sugar level. The mean of total glucose and of glucose above fasting level was calculated for each group and these values were also plotted semilogarithmically (Fig. 8, based upon numbers given in Appendix II, Table 3). It was then obvious that the best reproducibility of the line independently of the glucose level was obtained when the semilogarithmically plotted values of total glucose were used. There was, however, a slight deflexion of the line after 20–30 minutes, after which the blood sugar fall appeared to be somewhat slower as was also found by JENNY (1952), IKKOS & LUFT (1957), for example. This deflexion of the line is probably due to disappearance of the difference in the glucose concentration between the blood stream and other parts of the external space within which the free glucose had diffused. After this time the fall in the blood glucose concentration may be due to passage of the glucose from the external space to an intracellular phase from where the glucose is taken up by metabolic processes.

A marked deflexion of the semilogarithmic curve for the increment values was found in the group with the lowest blood glucose level. In view of this deflexion it is hardly possible to give the increment index a simple mathematical expression independent of the glucose level and suitable for the calculation of correlation coefficients.

Total index was therefore chosen to describe the rate at which the glucose passed into the intracellular phase. Owing to the above-mentioned deflexion, in the calculation of k it was decided to ignore the first 20 minutes of the curve. It is true that the total glucose curve does not reflect a monomolecular reaction, since it does not go towards 0, but it has the advantage that within the range under consideration it is roughly linear and the angle it subtends with the horizontal plane is largely independent of the glucose level.

Since rapid unexplained fluctuations have been observed in the blood glucose level (HANSEN, 1923, ANDERSON et al., 1956) it was considered more valuable to have two points on the smoothed curve

glucose given by the intravenous route and the oral route, respectively owing to a special sensitivity to the concentration of the glucose in the blood in the portal region (SCOW & CORNFIELD, 1954)

i. *Renal excretion of glucose* The loss of glucose in the urine following intravenous injection of 25 g glucose is normally insignificant because the blood sugar level falls rapidly below the renal threshold for glucose. Several investigators have found the amount of glucose excreted in the urine after injection of 25 g glucose to be less than 2 g (AMATUZIO *et al* 1953, IKKOS & LUFT 1957)

Neither will the amount of glucose lost with the urine generally affect the results of the test on oral administration of glucose However inter-individual differences in the renal threshold must be considered, which are probably of greater importance in the oral test where the blood sugar level is near the threshold level for a longer period.

j *Secretion of insulin and consequent increase of factors raising the blood sugar level.* The interval between the rise in blood sugar concentration and increased insulin activity is not known with certainty Evidence available has made is probable that the insulin promptly adjusts itself to the glucose level (ANDERSON *et al.*, 1956, METZ, 1960) It seems reasonable to expect an increased activity after a few minutes but a maximal effect not before 20—30 minutes after the initiation of hyperglycaemia. In diabetes the secretion of insulin is retarded (YALOW & BERSON 1960)

It is not properly understood how early increased insulin activity and consequent fall of the blood sugar level initiates homeostatic processes.

The oral test, in which the glucose is taken up by the body for a longer period and the rise in the blood sugar persists for a longer time than on rapid intravenous injection of glucose might be more sensitive to the effect of increased insulin secretion and other endocrine factors which do not exert their full effect in association with the very transient increase in the blood sugar following intravenous administration.

On the basis of these comparisons it appears likely that the more physiological oral test might have advantages regarding the demonstration of potential diabetics but that the rapid intravenous test, owing to the absence of factors influencing absorption and difficult to assess, is superior for describing the effect of non-endocrine factors, and particularly of constitutional influences, on the results of the tolerance test.

APPRAISAL OF INTRAVENOUS GLUCOSE TOLERANCE TEST

In the present investigation the intravenous tolerance test was chosen with the injection of 100 cc of 25% glucose. This technique has in recent years been widely used in routine clinical work and in experimental studies (HAMILTON & STEIN 1942, GREVILLE, 1943, AMATUZIO *et al* 1953, DUNCAN 1956 a, IKKOS & LUFT 1957 WEST & WOOD, 1959) The results are described by *inter alia* the rate at which the blood sugar falls after the rapid initial rise In order to determine this rate independently of the glucose level, the blood sugar values are plotted on semilogarithmic paper and then show a curve which is roughly linear and whose slope is taken as an expression of the rate of the glucose uptake by the tissues. This rate (k) can be expressed according to the general formula for a monomolecular reaction

Table 28. Classification of total index with correction for low glucose level.

Class	Uncorrected total index	Bls_{43} (mg./100 ml.) without steroid priming	with steroid priming
I	0—0.59		
II	0.60—0.89		
III	0.90—1.19	>130	>135
IV	1.20—1.49	125—129	130—134
V	1.50—1.79	120—124	125—129
VI	1.80—2.09	115—119	120—124
VII	2.10—	<115	<120

the blood sugar enhancing effect of steroids (see page 54). The assignment to a given class was decided by T. I. only when the blood sugar level at 43 minutes (bls_{43}) was higher than that given for the class, otherwise it was decided by bls_{43}. The correlation between bls_{43} and T. I. proved remarkably high (= 0.57)

CORRELATION BETWEEN THE GLUCOSE LEVEL AFTER INTRAVENOUS INJECTION AND THE SIZE OF THE SPACE IN WHICH GLUCOSE WAS INITIALLY DISTRIBUTED

The glucose level measured t certain time after intravenous injection can be regarded as proportional to the size of the extracellular space in which the glucose was initially distributed. To elucidate this and to record the dependency of the increase in the blood sugar level on the ratio between different types of tissue the urine sugar values as well as the blood sugar values after 20 and 43 minutes were used. Bls_{20} should presumably be more strongly correlated with the highest glucose level tha bls_{43}. The urine sugar alue ma correspond to the maximal glucose increase but its usefulness is limited by methodological inaccuracy and the varying renal threshold for glucose which was a probably insignificant but unknown source of error in the present investigation.

EFFECT OF GLUCOCORTICOID PRIMING ON GLUCOSE TOLERANCE TEST

Pretreatment with cortisone and cortisone-like substances has been used in recent years in association with the glucose tolerance test. Some authors have claimed that such pretreatment increases the possibility of detecting potential diabetics (for survey see Conn, 1958, Faj s & Conn, 1961)

Recently however the value of the method has been questioned by West (1960), for example who found such pretreatment to elevate the 2 hour value of the oral glucose test in persons whose two parents were diabetics not more than in persons without diabetes heredity Neither did Jackson (1961) find any definite increase in the incidence of hyperglycemic cortisone glucose tolerance tests in various types of persons in whom prediabetes might be expected, g mothers of children over weight at birth.

I the beginning of the present investigation it was intended to use hydrocortisone provocation in the entire material largely in accordance with the method described by Duncan (1956 b). Thus 150

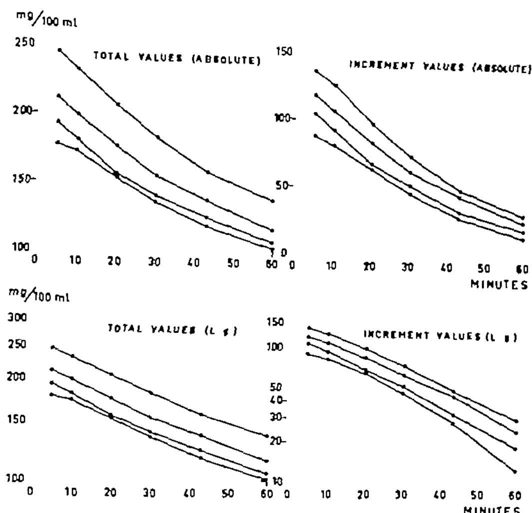


Fig 8. Curves based upon mean values of blood sugar concentration at different intervals after injection of glucose, expressed as total and increment values, respectively after division into quarters of the pilot series according to the height of the maximal glucose level.

accurately determined than a number of points with a lower degree of accuracy. Therefore several determinations were made of the glucose 20 and 43 minutes after the injection. The time of 43 minutes was chosen to simplify calculations because then the equation

$$k = \frac{2.3 (\log C_1 - \log C_2) \quad 100}{t_2 - t_1}$$

can be shortened to

$$k = 10 (\log C_{20\,min.} - \log C_{43\,min.})$$

The T L values obtained could not be used without correction for calculation of correlation coefficients. It was found that in a number of cases the blood sugar concentration during the period under consideration, i e 20—43 minutes after the injection of glucose had fallen to such low values that one might suspect the fall to be retarded by homeostatic factors. Such a retardation would cause too low an index value. For this reason a corrected classification was constructed (see Table 28), where allowance was also made for

Table 23. Classification of total index with correction for low glucose level.

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the blood sugar enhancing effect of steroids (see page 54). The assignment to given class was decided by T. I. only when the blood sugar level at 43 minutes (bls_{43}) was higher than that given for the class, otherwise it was decided by bls_{43}. The correlation between bls_{43} and T. I. proved remarkably high (= 0.87).

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In the beginning of the present investigation it was intended to use hydrocortisone provocation in the entire material largely in accordance with the method described by DUNCAN (1956 b). Thus 150

Table 29 Blood sugar levels without priming and various periods after priming with hydrocortisone or prednisolone

Interval after priming (hours)	Bls_{20} No priming n	mean (mg./100 ml.)	SD		Bls_{43} No priming n	mean (mg./100 ml.)	SD	
	145	164.5	22.1		144	128.2	21.2	
	150 mg. hydrocortisone		10 mg. prednisolone		150 mg. hydrocortisone		10 mg. prednisolone	
	n	mean (mg./100 ml.)	n	mean (mg./100 ml.)	n	mean (mg./100 ml.)	n	mean (mg./100 ml.)
2 —2½	1	154			1	111		
2½—3	2	144	6	164	2	125	6	118
3 —3½	12	165	6	153	12	125	6	121
3½—4	7	186	6	190	7	153	6	151
4 —4½	9	170	6	167	9	140	6	140
4½—5	2	181			2	139		
5 —5½	1	184			1	164		

mg hydrocortisone was given 2–4 hours before the beginning of the glucose tolerance test. The value of the method, however proved questionable, and it was abandoned after 34 tests. In a later group of 24 persons 10 mg prednisolone was given 2–4 hours before the glucose injection. In the selection of the latter less electrolyte-active preparation consideration was given to the possibility that the dose of hydrocortisone might have increased the size of the extracellular space during the first few hours after the injection and before the glucose increasing effect had started with the result that remarkably low blood sugar levels were obtained during the first 2–3 hours after the administration of hydrocortisone. Prednisolone however had largely the same effect as hydrocortisone but the smaller dose of steroid caused, as expected, a somewhat smaller increase in the blood sugar level.

The time of the maximal effect of steroids after oral administration was studied by determining the average glucose level at different intervals after the administration of steroids. This level was calculated as a mean of the glucose values noted 20 and 43 minutes, respectively after the glucose had been administered.

It is clear from Table 29 that full effect of the steroid on the blood sugar was probably not obtained until 3½ to 4 hours after administration. This interval agrees well with the curves of THORN, RENOLD & WINEGRAD (1957) and also with the maximum eosinophil depressive effect of these substances (WEST 1958).

The investigation would not allow of any conclusions as to WEST's (1959) statement that glucocorticoid priming causes the blood sugar to rise further until 4–6 hours after the administration of hormone.

METHODS

MEASUREMENT OF GLUCOSE IN BLOOD

The glucose-concentration in blood was determined according to Hagedorn–Jensen

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Steroid-preparations courteously supplied by Ferring AB Malmö.

(HAGEDORN HALSTRØM & NORMAN-JENSEN, 1935), on capillary blood from the finger tips.

The determination was made 4–8 hours after sampling. To prevent glycolysis sodium fluoride was added to the samples.

Each day double determinations were made on blanks containing only precipitating solution, as well as double titrations of the thiosulphate solution used against an exactly 0.05 iodide solution.

The order of the error of titration could be judged from the mean difference between the two determinations on the thiosulphat solution. The difference was equivalent to 1.7 mg./100 ml. of glucose. On double determinations of blanks mean difference of 3.3 mg./100 ml. was obtained, which was considered to be an expression of the total range of variation of laboratory methods.

On the first two examination days two capillary samples were collected, one of 0.1 and one of 0.05 cc., but the latter amount proved less suitable for the relatively low glucose values found. Therefore later 4 samples of 0.10 cc. each were collected at every interval. The 4 samples were collected within period of 2–3 minutes. In the calculation of the mean, the 3 values that were closest together were chosen in order to avoid the effect of strongly deviating values – in addition, for technical reasons, it was sometimes possible to obtain only 3 samples in the short time available for sampling. The values excluded deviated largely equally on both sides of the mean range.

In order to estimate the total error of the determination of the glucose level, the arithmetic mean was calculated of the 3 most similar values for each person and sampling time and then the mean of the deviations of the single observations from this arithmetic mean was calculated. The mean of these deviations, 3.1 mg./100 ml., was 14 % of SD of the blood sugar values calculated for the whole material. The corresponding figures for all 4 values were 4.1 mg./100 ml. and 17 % of SD respectively.

The samples were collected in 2–3 minutes. During these minutes the blood sugar fell. The size of the fall is indicated by the mean value after 20 minutes, for the first sample it was 166.1 mg./100 ml. while for the fourth sample it was 164.2 mg./100 ml. The corresponding values at 43 minutes were 18..0 and 180 0 mg./100 ml.

T void thrombophlebitis the patients received 10 000 I. U heparin together with the glucose, which has been shown not to affect the glucose values (DUNCAN 1956 a).

MEASUREMENT OF GLUCOSE IN URINE

The urine produced from immediately before the injection of glucose until 60 minutes afterwards was collected in one portion. It was examined by one and the same person with Tes-Tape "Lilly To check the reliability of these determinations the glucose in specimens from 85 consecutive samples was measured also polarimetrically (HAMMARSTEN 1955). The results are demonstrated in Fig. 9

RESULTS

T describe the results of glucose tolerance test in groups with varying risk of being a diabetes gene carrier cumulative frequency diagrams were constructed for the distribution of total index and blood sugar concentration (Fig 10). The

Courteously supplied by Vitrum, Stockholm.

Courteously supplied by Lilly and Co.

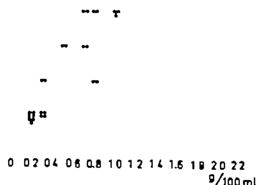


Fig 9. Comparison between Tes-Tape scores and urine sugar concentration as measured by polarimetric method.

figures on which these diagrams are based are given in Appendix II Table 4

The correlations between the results of the intravenous tolerance test and different somatic determinations are expressed as correlation coefficients (Table 30)

EFFECT OF NOT STRICTLY COMPARABLE STUDY-GROUPS ON THE RESULTS OBTAINED

Two of the subjects showed signs of shock during the intravenous test, which was therefore discontinued. In one case the psychic reaction was so intense that the test could not be performed. Two of the subjects had by mistake eaten breakfast on the morning of the examination so that the test could not be performed. In one case the blood sample obtained after 43 minutes was lost.

In the calculation of the correlations the glucose values from the entire material were used, besides which the values were divided into 3 subgroups, namely·

1. Values for which there was no reason to doubt their correctness (103 probands)
2. Values after glucocorticoid priming (58 probands)
3. Values from examination in which only 90 cc glucose solution was given (11 probands) as well as cases in which the injection was retarded owing to ruptured vessels (14 probands) and finally values from 2 examination days (16 probands) during which the mean glucose value was remarkably different from the mean of the entire material which could later be explained by subsequent analysis of the composition of the groups examined on these days.

As to the 3 subgroups bls was used for the comparisons. No substantial differences were found between the types of correlation coefficients obtained (Table 30)

DISCUSSION

GLUCOSE TOLERANCE IN ASSUMED CARRIERS OF THE DIABETES GENE

No correlation was found between the results of the glucose tolerance test independently of the method used for estimating, and the risk of being a carrier of the diabetes gene This is in disagreement with the findings of most earlier investigators (PINCUS & WHITE, 1934 WHITE, 1955 CONN 1958) though it should be observed that some evidence questioning the value of the glucose tolerance test for detecting prediabetic conditons is also available (UNGER, 1957 LAMBERT JOHNSON & PAUL, 1961 KLIMT *et al.* 1961), and with special reference to youths, MACKLER & FISCHER (1934) found no constant pathologic curves on an oral tolerance test among 30 sibs, aged 2–19 years, of juvenile diabetics The observation might thus be explained by the low age of the subjects examined. It is well known that the glucose tolerance decreases with increasing age (CHESROW & BLEYER, 1954 SILVERSTONE *et al* 1957) and the tolerance test may then be more applicable for tracing a prediabetic state

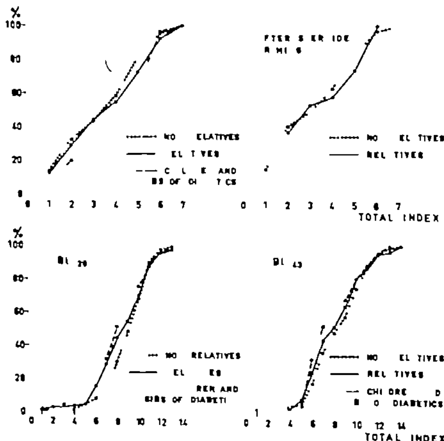


Fig. 10. Cumulativ diagrams of distribution of total index, and blood sugar concentration 20 and 43 minutes after glucose injection, among relatives and non-relatives of diabetics.

Neither can it be excluded that the method used with its unphysiological administration of glucose is not so good as the oral test for detecting prediabetic conditions. In this respect the longer period during which glucose is taken up on oral administration might be important. On intravenous administration with rapid injection of glucose the hyperglycaemic phase is so short that the endocrine mechanisms regulating the blood sugar concentration may not have time to react fully.

The investigation underlines that minor deviations in the results of the glucose tolerance test allow of no conclusions as to whether a person will develop diabetes or not. When the results of the tolerance test are correlated with the risk of being carrier of the diabetes gene it might be possible roughly to estimate the risk of diabetes development, but even then the considerable intra-individual variations of the results of the glucose tolerance test must be considered, as pointed out by FREEMAN *et al.* (1942) and others. It can-



Fig 9 Comparison between Tes-Tape scores and urine sugar concentration as measured by *polarimetric method.*

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between the significance of the different somatologic radicals certain differences were found. The strongest correlation was found between blood sugar levels and length-factor and sturdiness-factor respectively. The correlation between the glucose level and muscle-factor was lower throughout – possibly it was only indirect and might be explained by the strong positive correlation between muscle-factor and sturdiness-factor. It must, however, be borne in mind that estimation of muscle volume by dynamometric recordings is probably much less accurate than measurement of length-factor and sturdiness-factor.

No significant correlation was found between the glucose level and body fat.

In the evaluation of these correlations it should be remembered that the correlation between bodyweight on one hand and length- sturdiness- muscle- and fat-factors on the other were of the same order (Appendix III, Table 1).

The observation that the fluid space in which the glucose rapidly diffuses was correlated best with the fat-free volume can probably be explained by the relative decrease of the extracellular space found in obesity (LJUNGGREN, IKKOS & LUFT 1957, POBORSKI PETTERSSON 1957). It has also been suggested (LICHTFIELD 1959) that the hypertonicity of the extracellular fluid in hyperglycaemia is of significance. If that is the case one may imagine an increased transfer of intracellular fluid from the muscle cells than from the fat cells with the much smaller fluid phase of the latter.

The total index, at least if calculated as here on the fall in the glucose concentration occurring after the first 20 minutes, is due, above all, to the passage of the glucose from the external compartments to the intracellular space, where it is used up by metabolic processes. Since no significant correlations were found between total index and the somatologic radicals, the rate of this passage appears not to be dependent on the ratios between the different types of tissues. In the evaluation of these correlations it should be borne in mind that total index might also be dependent on factors not measured, such as glucose uptake by the liver and tending to mask any variation of total index with body build. Further, if the transport of the glucose through the cell wall is not owing chiefly to a glucose gradient across the cell membrane it might be less dependent on the ratios between the different types of tissue and the distribution of fluid.

In the assessment of the significance of the results obtained it should perhaps be recollected that the effect of the fat is said to depend on whether the latter is growing or stationary (BEAUDOIN *et al.* 1953). In view of the age of the persons studied in the present investigation it may be assumed that the fat was on the increase and should thereby tend to depress the level of the glucose tolerance curve.

The findings described above suggest that the dose of glucose used should be adjusted according to fat-free bodyweight if the result is to warrant any conclusions on the height of the sugar curve. The same opinion is expressed by WIERZUCHOWSKI (1938), WATT & WOOD (1959), and others. The formulae used for the calculation of fat-free weight are, however, less satisfactory and this is probably the main reason why the correlation between bh_{20} and total weight was numerically stronger than that between bh_{20} and fat-free weight. It may therefore be advantageous to adjust the dose according to total body weight except for very obese persons, for whom it might be better to estimate the fat-free weight.

Table 30. Coefficients of correlation between results of glucose tolerance test and various somatic data (For evaluation of significance See Appendix V).

	n	Bls_{20}	Bls_{Ω}	Bls_{Ω_1}	Bls_{Ω_2}	Bls_{Ω_3}	Urine-sugar	Total index
		203	202	103	58	41	190	[illegible]02
Bls_{20}	203		0.[illegible]9	0.74	0.74	0.67	0.18	—0.52
Bls_{Ω}	202	0.79					0.13	—0.87
Bls_{Ω_1}	103	0.[illegible]4					0.03	—0.86
Urinesugar	190	0.18	0.13	0.03				—0.00
Total index	202	—0.52	—0.87	—0.80	—0.81	—0.87	—0.00	
Weight	467	—0.34	—0.21	—0.13	—0.18	—0.42	—0.36	0.03
Fat free weight	208	—0.31	—0.22	—0.21	—0.29	—0.22	—0.31	0.07
Height	467	—0.28	—0.20	—0.14	—0.38	—0.21	—0.27	0.07
Circumf. chest	146	—0.39	—0.23	—0.26	—0.12	—0.46	—0.26	0.12
Tibia length	208	—0.24	—0.14	—0.10	—0.27	—0.10	—0.32	0.03
Radius length	208	—0.25	—0.13	—0.15	—0.13	—0.13	—0.33	—0.02
Condyl. breadth	208	—0.30	—0.25	—0.16	—0.25	—0.43	—0.33	0.14
Distyl. breadth	208	—0.22	—0.16	—0.25	—0.07	—0.05	—0.25	0.06
Handgrip	208	—0.17	—0.16	—0.20	—0.12	—0.15	—0.18	0.12
Shoulder thrust	206	—0.13	—0.11	—0.11	+0.02	—0.23	—0.00	0.08
Shoulder pull	206	—0.12	—0.13	—0.19	—0.01	—0.11	—0.16	0.15
Skinfold back	208	—0.09	—0.11	—0.08	—0.01	—0.16	0.00	0.12
Skinfold lat. chest	208	—0.09	—0.11	0.00	—0.01	—0.27	—0.04	0.10
Skinfold abdomen	208	—0.12	—0.11	—0.06	—0.01	—0.23	—0.03	0.12
Weight minus fat-free weight	208	—0.07	0.01	0.07	0.13	—0.22	—0.08	—0.01

[1] Refers to subgroups given on page 56.

not be excluded that the group with extreme values for the tolerance test contains a larger number of carriers of the diabetes gene *that will later develop the* disease, which would then suggest that the glucose tolerance test reflects factors increasing the penetrance of the disease.

Treatment 2–4 hours before the glucose tolerance test with 150 mg hydrocortisone or 10 mg prednisolone was not found to increase the value of the test *for diagnosing prediabetic conditions.*

DEPENDENCE OF THE INTRAVENOUS GLUCOSE TOLERANCE TEST ON BODY VOLUME AND BODY BUILD

On rapid intravenous injection of glu*cose the blood sugar* level appears to be initially dependent on the ratio between the amount of glucose injected and the volume *of fluid in which it is rapidly dis*tributed. Gradually however the blood sugar level is also influenced by the slower passage of glucose from this external space, which *may be largely identical with the* extracellular space (FRANCKSON, CONARD & BASTENIE, 1959) to the intracellular compartments. Whether this passage depends upon a pressure gradient from one phase to another or there exists a special transport system for glucose through the cell membrane *(PARK et al 1959) is not* known with certainty – it is probably a result of both factors being of different importance in different tissues.

The values considered dependent on the size of the external space namely the blood sugar levels at 20 and 43 minutes after injection of glucose and the amount of urinary sugar are negatively correlated with bodyweight, (table 30). On comparison

between the significance of the different somatologic radicals certain differences were found. The strongest correlation was found between blood sugar levels and length-factor and sturdiness-factor respectively. The correlation between the glucose level and muscle-factor was lower throughout – possibly it was only indirect and might be explained by the strong positive correlation between musle-factor and sturdiness-factor. It must, however, be borne in mind that estimation of muscle volume by dynamometric recordings is probably much less accurate than measurement of length-factor and sturdiness factor.

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IX DISCUSSION

OCCURRENCE AND PENETRANCE OF THE DIABETES GENE

The present investigation emphasized the conception of the hereditary nature of diabetes mellitus. The values observed fit in best with an autosomal recessive inheritance, though the results of the analysis are also to a certain degree compatible with a dominant gene of low penetrance. The material does not lend itself well to evaluation of the possibility of aberrant, numerically less important types of diabetes. As to the diabetes gene, however there appears to be no distinction between diabetes of chiefly juvenile and diabetes of chiefly adult onset The asthenic features ascribed to young diabetics are referable to the effect of the disease during childhood and adolescence.

Broadly speaking assuming autosomal recessive inheritance, manifestation occurs chiefly among homozygotes and then in only about 80 % In this respect, however the sexes differ in that the morbidity among elderly persons is higher in females than in males.

Under certain conditions heterozygotes may also develop the disease, and this may then help to explain a certain overmorbidity among groups in which the frequency of diabetes is definitely higher than the 4 % expected with a gene frequency of 0.2 and manifestation in homozygotes only

As an example of such groups, reference might be made to acromegalics in whom frank diabetes is said to occur with a frequency of about 25 % (For survey see MILLER, 1960). The diabetes in acromegaly can probably be explained by the assumption that the increase in growth hormone unduly stresses the beta cells, which successively become exhausted and which have been shown to develop degenerative and atrophic changes in different types of animals (For survey see YOUNG & KORNER, 1960)

It seems probable that also other blood sugar enhancing factors, *e g* cortisone administered, or produced in increased amount indirectly stimulate the beta cells to an increased production of insulin, which may result in an undue stress on the insulin producing apparatus.

A stress of endocrine origin might be of significance in the causation of the increased frequency of diabetes among women with many children and possibly also among women who have several abortions.

If the insulin antagonistic activity becomes sufficiently intense even persons without the diabetes gene might develop diabetes, particularly if the function of the pancreas is impaired by some inflammatory or vascular lesion, for example But it seems reasonable to suppose that this risk of developing diabetes is greater even if the diabetes gene occurs *only in a single* dose Only when the gene occurs in a double dose however *will an insulin antagonistic* activity within the normal biologic

range cause the penetrance of the genotype.

In the absence of pronounced pathological conditions, factors favouring penetrance seem to be obesity and as fa as women are concerned reproduction. But it cannot be concluded that this relationship is causal and it cannot be excluded that social environments or endocrine influences may contribute to the correlations.

RELATION BETWEEN CONSTITUTION AND PENETRANCE OF THE DIABETES GENE

The investigation showed that certain tendency to overweight t the 18 year level occurs before the manifestation of diabetes in young males. This overweight may be related to the early sexual development of prediabetic children. As pointed out by several investigators (for survey see TAN NER, 1955) early maturers are not only persons whose growth is advanced at all ages. Also as adults they have more weight for height than late maturers. Children and youths who develop diabetes appear to fit in well with this picture.

A close correlation exists between the rate of sexual maturation and growth. Thus, the chief signs of sexual maturation, i e menarche in the female and corresponding genital development in the male, is generally preceded by accelerated linear growth, the increase being greatest 0.5 to 1.5 years before these occurrences, i. e at 11 years in girls and 18 years in boys (For survey see TANNER, 1955) This period of increased endocrine activity is accompanied by higher frequency of the diagnosis of diabetes with peak frequency some 2 years earlier in girls tha in boys, i. e coinciding largely with the period of accelerated linear growth in both sexes, which has been pointed out by SECKEL (1960) nd others.

Though psychological factors may be of significance in the explanation of the connection between puberty and the increased frequency of manifest diabetes, possible diabetogenic effect of various growth promoting factors active in puberty must be considered. The most important of such factors seem to be the pituitary growth hormone and various androgenic agents. While some uthors claim that the steroid growth factor is the chief initiator of acceleration of skeletal growth at puberty (KINSELL, 1954) others propound that the pituitary growth hormone is of greater importance in respect to linear growth (SECKEL, 1960).

An increase in the production of 11-oxy steroids with their well known diabetogenic effect is probably of less importance since its protein catabolic effect does not appear to gree with the observed increase in skeletal and muscular growth. The possibility must, however be emphasized that though the acceleration of growth and the increased penetrance are parallel, they are not necessarily of uniform origin.

Summarizing, a common endocrine background of juvenile diabetes cannot be concluded with certainty but the hypophyseal growth hormone and possibly androgenic substances are probably of importance. It seems reasonable to assume that these hormones also in the following decades favour penetrance, and explain the increased frequency of the disease in males up to the age of 50 years. This sex difference, however may also to some extent be due to the possibility that endocrine factors in females of fertile age tend to counteract penetrance of the disease and thereby prevent its development until the menopause.

In animal experiments the diabetogenic effect of the pituitary growth hormone seems to be well documented (HOUSSAY & BIASOTTI, 1931 HOUSSAY & ANDERSON,

1949 Young, 1937 Cotes, Reid & Young, 1949) and in human diabetics increased values for the growth-hormone have been found (Fordman & Gemzell, 1960 Ehrlich & Randle, 1961)

CAUSE OF RETARDATION OF GROWTH IN JUVENILE DIABETES

An increasing number of juvenile diabetics have a clinical picture of total diabetes with cessation of insulin production within the first few years of the disease (Wrenshall, 1960) and a corresponding increase in the requirements for exogenous insulin and difficulty in control of the disease According to White (1960), by the fifth year 90 % of diabetic children show such a totally diabetic state.

This has been confirmed by histological findings made in the pancreas of diabetics aged 1–29 years (Maclean & Ogilvie, 1959) that in those in whom the disease had run an acute course with death supervening within a matter of a few weeks, the average size of the islets was greater than in a group of control subjects. In contrast, the pancreata from those patients with a more chronic disease course contained islets significantly smaller than in the controls

The retardation of growth seems to be more pronounced if the disease becomes manifest before the period of rapid growth – about the age of 13 years in boys – and may be due to lack of production of insulin with consequent reduction of the effect of pituitary growth hormone or possibly testosterone on the puberal growth spurt.

It appears that both these hormones require the presence of insulin to exert their anabolic effect. In animal experiments *growth hormone has been shown to lack anabolic effect in the absence of insulin* (Milman de Moor & Lukens, 1951 Scow & Chernick, 1960) Testosterone has been shown to have no depressing effect on nitrogen excretion in pancreatectomized female dogs (Sirek & Best 1953)

The present state of our knowledge will not allow of any discussion of the possibility that the lack of insulin *per se* might contribute to the retardation of growth. *Insulin has, however been shown to have an* N retaining effect also in hypophysectomised animals (Salter & Best 1953, Lukens, 1958)

Evidence is available for the assumption of reduced androgen production after the young male had diabetes for some years (Bergquist 1954 b) with consequent impairment of sexual as well as muscular development In the treatment of diabetic dwarfism testosterone seems to be most effective (White, 1959)

The retardation might also occasionally be ascribed to dietary restrictions during the years of growth.

CAUSE OF OVERWEIGHT OF NEW BORNS OF FUTURE DIABETICS

Inherited growth factors might be the cause of the tendency to overweight at birth of children of women as well as men who later in life become diabetic. Since the birth of several children appears to favour penetrance considerably the more marked increase when the mother develops diabetes can be explained partly by the overweight, at birth, of children of high birth number

RELATION BETWEEN GLUCOSE TOLERANCE CURVE AND RISK OF DEVELOPING DIABETES

The result of the glucose tolerance test may be regarded largely as reflecting a certain endocrine situation. *It may be*

assumed that the curve is often elevated by diabetogenic factors, but that in conditions with a strong secondary production of insulin, the curve is even depressed as long as the beta-cells are intact. If hyper activity is followed by state of exhaustion, the glucose tolerance curve may be pathologically elevated during a period before the appearance of manifest diabetes. This period may be longer among elderly people.

Concerning young males, the result of the intravenous glucose tolerance test with and without glucocorticoid priming did not appear to be related to the risk of being a carrier of the diabetes gene. However it cannot be excluded that the group with the highest glucose curves includes an increased number of such gene carriers who will develop diabetes, which would confirm that the glucose tolerance curve reflects factors increasing penetrance. This can only be proved by a longitudinal study which is being planned.

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SUMMARY

Attempts were made to trace all male diabetics between the ages of 17 and 25 years in a well defined geographical area and all 18 year old male relatives of dia betics in that area.

The probands and age-matched controls were studied from various angles in order to obtain data to elucidate various genetical and constitutional aspects of diabetes mellitus.

The following observations were made·

GENETIC-STATISTICAL ANALYSIS

a. Diabetes occurred with increased frequency among relatives of juvenile dia betics and the distribution of the disease was compatible with the assumption of a uniform diabetic gene.

b An autosomal recessive mode of inheri tance seemed to be the most likely transmission of diabetes mellitus Then the mutant allele would occur at about 30 % of the general population of the investigated area. Penetrance would occur during lifetime in about 70 % of male and 90 % female homozygotes. If pene trance occurs among heterozygotes it appears to be low

c. Dominant inheritance seemed to be less likely but could not be excluded. The proportion of pathogenic alleles can then be calculated to be of the order of 0 05 with penetrance among about 25 % in male and 30 % in female gene carriers.

d. The possibility of different genotypes manifesting the same or similar clinical picture could not be exluded but appeared less likely If a main genotype be assumed the occurence of aberrant genotypes with similar manifestations of the disease must be low

GROWTH AND DEVELOPMENT OF DIA BETICS AND ASSUMED CARRIERS OF THE DIABETES GENE

a. Children and sibs of diabetics at 13 about 18 and about 50 years of age tended to be taller and heavier than the controls.

b *Attempted somatologic differentiation* regarding the cause of overweight at 18 years of children and sibs of dia betics suggested an increase not only of height but also of skeletal sturdiness and fat free weight.

c. 18 years old males who developed diabetes within the following 5 years tended to be heavy for height. The lack of strictly comparable controls prevented satisfactory somatologic differentiation — though certain evidence was available for the assumption of increased skeletal sturdiness and muscular strength.

d. Among males who developed the disease before 13 years, at 18 years a certain reduction in weight, height and skeletal sturdiness as well as muscle strength was noted. It could not be decided whether growth was inhibited in males who developed the disease between 13 and 18 years.
e. The amount of subcutaneous fat in juvenile diabetics tended to increase with the duration of the disease
f. At 50 years of age the diabetic was overweight independent of the duration of the disease

BIRTHWEIGHT OF DIABETICS AND ASSUMED CARRIERS OF THE DIABETES GENE

a. Female diabetics at the 50 year level and 70 year level had 1–2 children more than male diabetics and non-diabetic women of corresponding ages.
b. Children of prediabetic mothers had birthweight of on the average 400 g more than normal. A corresponding increase of about 150 g. was observed when the father was found to develop diabetes, and an increase of about 100 g. when the proband himself had developed diabetes before the age of 25 years. An increase of about 100 g. was also found for the sibs of the young diabetic probands.
c. Overweight of the proband at birth when the mother later developed diabetes may be ascribed partly to an increase of weight with increasing birth number. This increase in weight was calculated to be about 100 g.
d. A certain overweight when the mother developed diabetes might also be ascribable to the mothers overweight which was found to be correlated with the birthweight of her children.

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INTELLECTUAL ENDOWMENTS OF YOUNG DIABETICS AND ASSUMED CARRIERS OF THE DIABETES GENE

a. The intelligence of juvenile diabetics was found to be somewhat, but not significantly higher than normal and did not differ with the duration of the disease.
b. No such higher degree of intelligence was found among relatives of the diabetics.
c. Diabetics was found more often in the higher socio-economic classes.

INTRAVENOUS GLUCOSE TOLERANCE TEST

A glucose tolerance test was performed by rapid intravenous injection of 100 cc of 25 % glucose solution. The results were judged by the rate at which the glucose level in the blood fall, expressed as total glucose concentration during the period 20–43 minutes after the injection of the glucose. this rate was called total index.

The following observations were made:

a. Total index did not appear to be related to an increased risk of developing diabetes, as judged from genetic calculations.
b. Total index did not appear to be correlated with certainty with different somatic measurements.
c. Administration of hydrocortisone or prednisolone 2–4 hours before the injection of glucose appeared to raise the level of blood-glucose but not to affect the ratios between the distribution of total index among relatives of diabetics compared with non-relatives.
d. The height of the blood glucose curve after intravenous injection of glucose did not seem to be related to the increased risk of developing diabetes owing to relationship with diabetic.

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APPENDICES

I—V

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The investigation added evidence for the inheritability of diabetes mellitus. The *disease appears to be caused by a uniform* mutant gene with a frequency of 0 17 to 0.20 in the population studied and then develops mainly in homozygotes. In these the frequency of manifestation increases with age but does not become 100% In creased growth and, as far as women are concerned, increasing number of pregnancies favour manifestation. In 20 year old male carriers of the diabetes gene, the glucose tolerance as judged by rapid intra venous injection of 25 g. glucose is not decreased.

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The present investigation was started at *the Medical Clinic of Kristianstad* and con tinued at the Medical Clinic, Malmö

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I feel indebted to Professor C. E. Quensel *for invaluable help with the statistical treat* ment of the data, and to Docent Jan Agrell, Head of the *Militärpsykologiska Institutet*, Stockholm for enabling utilization of data *on intelligence tests of recruits*.

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Acknowledgement for smooth co-opera tion is also due to members of the Swedish Armed Forces and technical assistants.

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Appendix I.

Table 1. Weight and height at induction in series B (subgroups I—VI described on page 9).

			Examined by Author	Not examined by Author I	II	III	IV	V	Total	VI
D			209	154	44	32	18	10	467	8
	weight		64.4	63.6	64.7	65.9	62.2	62.5	64.3	
		SD	7.5						SD 7.6	
	height		175.5	176.2	175.4	176.5	174.3	172.9	175.8	
		SD	6.5						SD 6.5	
Br	n		106	78	23	16	8	6	237	
	weight		64.8	63.7	65.0	68.3	63.7	62.8	64.5	
	height		175.8	176.4	176.2	181.3	175.4	173.3	176.3	
Bc	n		103	76	21	16	10	4	230	8
	weight		64.2	63.5	64.4	63.6	61.5	62.3	64.0	
	height		174.9	176.0	174.5	171.7	173.7	172.3	174.9	

Appendix I

Table 3. Distribution of occupations of fathers, Series A and B.

Father's occupation \ Series	A: 6th reg. distr	A: 7th reg. distr	A: Total	Br	Bc
Agriculture	17 28.3 %	7 14.3 %	24 22.0 %	25 23.6 %	20 19.4 %
Farm labourers	2 3.3 %	3 6.1	5 4.6 %	3 2.8 %	5 4.9 %
Industry workers	12 20	11 22.4 %	23 21.1 %	27 25.5 %	32 31.1 %
Salaried employees, foremen	7 11.7 %	8 16.3 %	15 13.8 %	15 14.2 %	12 11.7 %
Unskilled labourers	12 20	6 12.2 %	18 16.5 %	5 4.7 %	4 3.9 %
Commerce	2 3.3 [illegible]	5 10.2 %	7 6.4 %	8 7.5 %	5 4.9 %
Professional employees	5 8.3 [illegible]	2 4.1 %	7 6.4 %	12 11.3 %	3 2.9 %
Employers	3 5 [illegible]	4 8.2 %	7 6.4 %	9 8.5 %	12 11.7 %
Not known	0	3 3.6 %	3 2.8 %	2 1.9 %	10 9.7 %

Appendix I

Table 4. Distributions and meanweights of parents of probands in series B according to their socio-economic level. (Division into social groups described page 17.)

Sex	Social group	Series Br: Diabetes known in: Parent or sib	Series Br: Diabetes known in: Grandparent, uncle, aunt or cousin	Series Bc	Weight series B mean (kg)
Female	I	1 (4 %)	8 (10 %)	4 (4 %)	62.5
	II	5 (23 %)	32 (38 %)	20 (21 %)	68.1
	III	16 (73 %)	44 (52 %)	72 (75 %)	66.6
Male	I	1 (4 %)	8 (10 %)	4 (4 %)	77.3
	II	6 (23 %)	30 (38 %)	19 (21 %)	75.9
	III	15 (73 %)	42 (52 %)	69 (75 %)	78.9

Appendix I.

Table 2. Distribution of occupations, Series B.

Occupation		Diabetes in parents or sibs	Series Br: Examined by Author	Series Br: Not examined by Author	Total Br	Series Bo: Examined by Author	Series Bo: Not examined by Author	Total Bo	Total B
Total	n	53	106	131	237[1]	103	127	230	407
	weight		64.5	64 6	64.5	64.2	63.7	64 0	64.3
	height		176 0	176.6	176.3	174.6	175.0	174.9	175.6
Agriculture		0([illegible] %)	21	16	37([illegible] %)	17	14	31([illegible] %)	68
			64.4	65 8	65.0	63.5	63.1	63 4	64 2
			174 8	176.7	175.6	173.0	174 0	173.5	174 6
Industry		10([illegible] %)	24	26	50([illegible] %)	39	38	77([illegible] %)	127
			64.6	64.0	64 3	61 7	60.3	62.1	62.9
			175.8	174.8	175.3	174 9	172.0	173.0	174 5
Commerce		7([illegible] %)	15	15	30([illegible] %)	15	16	31([illegible] %)	61
			65.2	60 4	62.8	61 6	61.6	61 6	62.2
			176.8	176.1	176.5	175 7	171.8	175.2	175.8
Miscellaneous		12([illegible] %)	18	24	42(17 4 %)	17	12	20([illegible] %)	71
			63.1	61 8	62.1	63.9	63.0	63.0	62.9
			173.0	174.5	174.2	173.9	173.0	173.9	174 1
Handicraft		6([illegible] %)	10	9	19([illegible] %)	9	14	23(9 7 %)	12
			59 6	66.3	62.8	67 0	63 6	65 0	61 0
			176.6	178 2	177 4	175.0	174.6	174.8	170 0
Student		12([illegible] %)	18	41	59([illegible] %)	6	33	39([illegible] %)	98
			67.8	67.5	67.6	66.8	66.3	66 4	67 1
			178.5	178.8	178.7	176.5	178.2	177 9	178 4

[1] If Br is corrected t match Bo, weight will be 64 1 kg. and height 176.2 kg.

Distribution of classes

1	5	13	14	24	19	15	9	7	5	0	2	1												
2	1	4	9	17	20	18	10	11	11	6	3	2	1	1	1									
3	1	5	22	34	57	73	78	59	55	43	21	5	7	3	0	0	1	0	1					
4	3	7	23	46	79	69	81	67	63	17	18	3	1											
5	1	1	7	12	21	33	37	31	28	18	9	8	1	0	1									
6	1	0	1	3	5	13	10	22	31	36	25	18	15	18	7	2	0	0	1					
7	1	3	6	21	18	28	39	41	30	12	8	1												
8	2	3	4	8	8	21	20	30	19	26	31	11	15	5	2									
9	1	1	1	6	5	16	22	18	24	22	14	6	5	2	1	1	0	1						
10	1	6	13	20	41	63	46	11	3	1														
11	3	5	3	11	25	22	29	23	32	14	18	7	11	2	3									
12	1	3	6	10	7	12	23	18	19	21	13	22	15	8	8	3	6	4	4					
13	4	5	11	11	17	22	30	22	13	19	18	20	9	1	0	2	0	1	0	1				
14	10	48	58	44	25	6	3	8	0	4	1	0	1											
15	14	67	56	42	21	8	3	2	2	2	1													
16	11	50	63	37	18	9	5	2	2	1	4	3	1	0	1	0	1	0	1	1				
17	3	3	5	14	4	13	16	22	18	28	8	13	17	10	11	5	4	5	1	3	1	2	1	
18	3	1	5	15	21	17	27	25	30	24	16	9	8	6	2									
19	52	45	30	19	19																			
20	1	3	8	25	24	47	19	37	17	18	5	3	0	1										
21	1	0	5	8	36	19	56	31	18	3	1													
22	6	3	4	9	17	11	13	21	17	7	4	6	7	4	1	0	1							
23	5	6	13	21	39	55	92	54	37	27	12	4	2											
24	6	5	19	15	63	51	36	35	15	9	3	1	1											
25	3	2	3	10	25	50	60	44	26	9	7													
26	1	9	13	21	33	58	32	37	53	37	19	22	7	14	3	11	1	1	1	1	0	0	0	1
27	18	33	52	80	79	63	43	16	0	0	0	0	1											
28	1	0	2	7	20	18	25	46	43	57	19	34	15	21	12	5	5	1	2	1	0	1		
29	1	3	2	24	40	68	78	56	34	24	17	6	1	0	1									
30	1	2	29	50	130	80	45	16	3															
31	3	11	23	34	27	34	36	32	29	23	27	19	18	10	11	8	8	4	3	2	3	1	6	
32	14	41	44	60	46	77	60	25	6	2														
33	31	39	80	87	51	43	14	8	2	2														
34	19	53	54	41	42	85	49	31	8	1														
35	40	50	74	79	68	37	14	10	5															
36	43	107	103	58	35	26	22	6																
37	10	71	64	85	121	66	35	18	5															
38	42	95	70																					
39	84	101	22																					
40	272	84	56																					
41	132	67	13																					
42	87	50	64																					
43	11	20	19	14	2	0	4	18	8	1	5	17	0	9	19	18	10	10	2	9	3	9	2	
44	1	1	1	2	2	17	20	33	29	36	40	14	5	0	0	2								
45	1	1	1	1	9	31	36	21	22	35	19	17	3	4										
46	0	0	1	0	5	13	21	12	14	20	9	5	1	2										
47	0	6			1	9	10	5	8	9	8	9	1	6										
48	1	1	0	1	3	9	4	4	2	6	2	3	1	2										
49	40	26	60	65																				
50	22	48	35	42	28	17	3	3																
51	28	34	26	27	33	30	13																	

Appendix II

Table 1 Characteristics of series II

		n	Mean-value	SD	Unit	Class-breadth	Lowest class-breadth
1	Weight at 13	114	43.7	0.8	kg	3.0	31 0—33.9
2	Height at 13	114	155.1	8 1	cm	3	138—140
3	Weight at induction	467	64.3	7 5	kg	3.0	45 0—47.9
4	Height at induction	467	175.0	6.5	cm	3	158—160
5	Tibia length	208	39 0	2.8		1 0	32.0—32.9
6	Radius length	208	25.8	1 4		0.5	21.0—21 4
7	Condylar breadth	208	9.56	0.42		0.2	8.3—8.4
8	Bistyloid breadth	208	5.82	0.30		0.1	5 0
9	Circumf. chest	146	90.2	5.3		2	4— 5
10	Shoe-size	204	42.1[1]	1.5[2]	Sw size	1	37
11	Handgrip	208	41 1	5.8	kilopond	2	2[illegible]—28
12	Shoulder thrust	206	55.9	11.8		3	29—31
13	Shoulder pull	206	34 1	6.8		2	[illegible]0—21
14	Skinfold back	208	6.	1.2	mm	1	3
15	Skinfold lat. chest	206	5.1	2.1		1	3
16	Skinfold abdomen	203	6.0	2.3		2	3—4
17	Weight min. fat free weight	208	11 6	0 1	% of weight	2	-8 – 7
18	Fat free weight	208	59 1	5.8	kg	2	44—45
19	Hair-growth chest	208	2.2	1.3	See Lindegård (1956)		1
20	Blood-pressure systolic	206	126	11	mm Hg	5	95—99
21	Blood-pressure diastolic	208	81	6		5	50—54
22	Pulse-rate	131	67 1	10.0	beat min	3	46—48
23	Birthweight	369	3.54	0.57	kg	0.25	2 00—2.25
24	Birthlength	249	51.2	2.2	cm	1	46
25	Sibs birthweight	229	3.57	0.47	kg	0.25	2.00—2.25
26	Mother's weight	376	68.9	14 7		3 0	45.0—47.9
27	Mother's height	374	164.2	5.3	cm	3	152—154
28	Father's weight	345	78.6	9 6	kg	3.0	51 0—53.9
29	Father's height	352	1 5 0	6.2	cm	3	155—157
30	Mother's age at 1st menst	356	13.8	1.3	year	1	10
31	Mother's age at 1st partur	374	25.3	4 7		1	17
32	Number of father's sibs	374	4 6	2.0	number	1	0
33	pat. cousins	359	4 0	1 8		= number of class	0
34	mother's sibs	384	4.5	2.1		1	0
35	mat. cousins	365	3.8	1 8		= number of class	0
36	sibs	398	2 4	1 7		1	0
37	Mean score intel. test	465	4 49	1 75	see page	15	1
38	Manual labour	207	2.1	0.7		17	1
39	Intellectual work	207	1 7	0.6			1
40	School-education	412	1.5	0.7			1
41	Socio-economic level	202	2.5	0 6			1
42	Urbanization	203	1.9	0.9			1
43	Days after enlistment	208	21.8	14 0	day	2	0—1
44	Bls[illegible]	203	164.5	22.1	mg /100 ml	10	80—89
45	Bls[illegible]	202	128.2	24.2		10	50—59
46	Bls[illegible]	103	12[illegible]	21 4		10	50—59
47	Bls[illegible]	58	132.7	22.0	"	10	50—59
48	Bls[illegible]	41	122.5	20 6		10	50—59
49	Urine sugar	190	2.6	1.2	Tes Tapescore		1+
50	Total index without correction	202	3.4	1 [illegible]		0.25	0.35—0.59
51	Total ind x corrected	202	3.9	1 9		See t bl 28	

[1] = 28 cm. r English size 8 [2] = English size 1.3

Appendix II

Table 4. Distribution of relatives and non-relatives in series B on total index, bls_{30} and bls_{0}- (cf Fig. 10).

a = with glucocorticoid priming   b = without priming   a + b

Total-index (classes given in table 23)	Non-relatives			Relatives			Children + sibs of diabetics		
	a	b	a + b	a	b	a + b	a	b	a + b
1	6	8	14	—	11	11	—	2	2
2	9	10	19	7	8	15	2	1	3
3	3	8	11	3	10	13	1	3	4
4	5	11	16	2	8	10	—	3	3
5	7	15	22	3	13	16	—	4	4
6	5	10	15	6	12	18	4	4	8
7	1	4	5	—	7	7	—	1	1

Bls_{30} (mg./100 ml.)	Non-relatives			Relatives[1]			Children + sibs		
	a	b	a + b	a	b	a + b	a	b	a + b
80—89					1	1			
90—99					1	1			
100—109							1		1
110—119		1	1		1	1			
120—129					1	1			
130—139	2	5	7	2	8	10	1	5	6
140—149	2	5	7	1	11	12		1	1
150—159	4	12	16	4	11	15	2	3	5
160—169	8	11	19	1	8	9		3	3
170—179	7	13	20	5	9	14	1	2	3
180—189	9	13	22	4	13	17	2	1	3
190—199	2	6	8	3	5	8		2	2
200—209	1	1	2		2	2		1	1

Bls_{0} (mg./100 ml.)	Non-relatives			Relatives			Children + sibs		
	a	b	a + b	a	b	a + b	a	b	a + b
50—59					1	1			
60—69		1	1						
70—79		1	1						
80—89					1	1			
90—99	1	2	3		5	5		1	1
100—109	4	9	13	3	12	15	3	4	7
110—119	6	13	19	4	13	17	1	4	5
120—129	3	9	12	2	5	7			
130—139	2	8	10	3	8	11		4	4
140—149	6	11	17	2	13	15		3	3
150—159	7	6	13	2	4	6	1	1	2
160—169	5	4	9	3	5	8	1	1	2
170—179	1	2	3	1		1	1		1
190—199		1	1		3	3			

[1] Except for first cousins of diabetics.

Appendix II

Table 2.

Age in years	Penetrance in per cent of P_{max} for respective sex at different ages: Males, Kristianstad	Males, Birmingham	Females, Kristianstad	Females, Birmingham	Penetrance among males in per cent of P_{max} for females (Kristianstad): Uncorrected for sex ratio	Corrected for sex ratio	Sex ratio 1952, Kristianstads län
4	3.2	0.8	2.8	0.4	2.5	2.3	1.05
9	6.0	2.8	4.0	1.2	4.6	4.1	1.06
14		4.7		2.7			
19	13.3	8.0	6.8	4.1	10.1	9.6	1.06
24		11.0		5.2			
29	18.9	15.0	9.2	7.1	14.4	13.	1.03
34		19.3		9.1			
39	28.5	24.3	11.7	11.3	20.2	19.5	1.02
44		30.8		15.5			
49	37.7	40.6	19.3	22.4	28.8	28.0	1.01
54		52.5		35.0			
59	56.6	65.0	45.7	50.2	43.2	43.1	0.95
64		78.1		67.1			
69	80.3	87.6	78.8	82.4	61.3	63.2	0.90
74		91.6		92.8			
79	97.6	98.7	97.5	98.5	74.5	78.0	0.86
84		99.9	98.7				
89	100.0	100.0	100.0	100.0	76.4	80.8	0.81

Appendix II

Table 3. Mean values of blood sugar expressed as total and increment values respectively in the "pilot series" divided into quarters according to the highest blood sugar level (cf. Fig. 8).

Time after zero (min)	I	II	III	IV
Total glucose (mg./100 ml.)				
5	177.7	193.0	211.7	215.2
10	172.7	181.1	199.4	232.6
20	153.2	155.9	175.9	206.5
30	135.2	139.9	161.6	182.6
43	117.2	123.9	136.5	157.6
60	101.2	105.9	115.4	137.6
Increment glucose (mg./100 ml.)				
5	88.9	105.3	119.3	137.3
10	82.0	93.4	107.8	126.5
20	64.4	68.2	84.2	95.6
30	46.3	52.2	62.9	71.5
43	28.3	32.9	41.9	49.7
60	12.4	16.2	21.2	20.8

	Weight	Height	Weight at 13	Height at 13	Birth weight	Birth length	Birth weight of sibs	Mother' weight	Mother height	Father' eight	Father' height
Shoe size	0.81([illegible])	0.73([illegible])	0.1?([illegible])	0.34([illegible])	0.30([illegible])	0.33([illegible])	0.18 ([illegible])	0.11([illegible])	0.35([illegible])	0.7?([illegible])	0.30([illegible])
Weight at 13	.35([illegible]) 0.58 (40)	0.49([illegible]) 0.53 (40)		0.72([illegible]) 0.80 (40)	0.33 ([illegible]) 0.19 ([illegible])	0.34 ([illegible])	0.33 ([illegible]) 0.10 ([illegible])	0.18 ([illegible]) 0.11 (33)	0.26 ([illegible]) 0 1 ([illegible])	0.17 ([illegible]) 0.28 (33)	0.01 ([illegible]) 0.18 (33)
Height at 13	0.30([illegible]) 0.65 (40)	0.53([illegible]) 0.78 (40)	0.72([illegible]) 0.80 (40)		0.32 ([illegible]) 0.16 ([illegible])	0.34 ([illegible])	0.30 ([illegible]) 0.28 ([illegible])	0.14 ([illegible]) 0.12 (33)	0.21 ([illegible]) 0.20 (33)	0.11 ([illegible]) 0.20 (33)	0.02 ([illegible]) 0.20 (33)
Birth weight	0.29([illegible]) 0.33 (78)	0.25([illegible]) 0.25 (78)	0.33 ([illegible]) 0.19 ([illegible])	0.32 ([illegible]) 0.16 ([illegible])		0.79([illegible])	0.52([illegible]) 0.52 (43)	0.13([illegible]) 0.16 (78)	0.17([illegible]) 0.19 (78)	−0.06([illegible]) 0.07 (78)	−0.04([illegible]) 0.02 (78)
Birth length	0.33([illegible])	0.29([illegible])	0.31 ([illegible])	0.34 ([illegible])	0.79([illegible])		0.37([illegible])	0.21([illegible])	0.24([illegible])	0.05([illegible])	0.03([illegible])
Sibs birth eight	0.29([illegible]) 0.21 ([illegible])	0.30([illegible]) 0.45 ([illegible])	0.23 ([illegible])	0.30 ([illegible])	0.32([illegible]) 0.52 (43)	0.37([illegible])		0.15([illegible]) 0.32 (43)	0.25([illegible]) 0.31 ([illegible])	0.00([illegible]) 0.00 ([illegible])	0.03([illegible]) 0.21 ([illegible])
Mother weight	0.17([illegible]) 0.19 ([illegible])	0.15([illegible]) 0.16 ([illegible])	0.18 ([illegible]) 0.11 (33)	0.14 ([illegible]) 0.13 (33)	0.13([illegible]) 0.16 (78)	0.21([illegible])	0.15([illegible]) 0.32 (43)		0.33([illegible]) 0.36 ([illegible])	0.07([illegible]) 0.08 ([illegible])	0.07([illegible]) 0.16 ([illegible])
Mother's height	0.27([illegible]) 0.06 ([illegible])	0.38([illegible]) 0.28 ([illegible])	0.26 ([illegible]) 0.01 (33)	0.21 ([illegible]) 0.20 (33)	0.17([illegible]) 0.19 (78)	0.24([illegible])	0.25([illegible]) 0.21 ([illegible])	0.33([illegible]) 0.36 ([illegible])		0.22([illegible]) −0.01 ([illegible])	0.22([illegible]) 0.11 ([illegible])
Father' eight	0.22([illegible]) 0.20 ([illegible])	0.33([illegible]) 0.17 ([illegible])	0.17 ([illegible]) 0.21 (33)	0.11 ([illegible]) 0.20 (33)	−0.06([illegible]) 0.07 (78)	0.05([illegible])	0.00([illegible]) 0.00 ([illegible])	0.07([illegible]) 0.08 ([illegible])	0.22([illegible]) −0.01 ([illegible])		0.46([illegible]) 0.47 ([illegible])
Father' height	0.17([illegible]) 0.38 ([illegible])	0.39([illegible]) 0.34 ([illegible])	0.01 ([illegible]) 0.18 (33)	0.03 ([illegible]) 0.30 (33)	−0.04([illegible]) 0.22 (78)	0.05([illegible])	0.03([illegible]) 0.31 ([illegible])	0.07([illegible]) 0.16 ([illegible])	0.22([illegible]) 0.11 ([illegible])	0.46([illegible]) 0.47 ([illegible])	

1 or evaluation of significance, See Appendi V

Appendix III

Table 1 Correlation coefficients between various somatological factors

Upper rows — Series B. Lower rows — Series A

Bracketed numbers indicate pairs of observation

	Weight	Height	Weight at 13	Height at 13	Birth weight	Birth length	Birth weight of sibs	Mother's weight	Mother's height	Father's weight	Father's height
Fat free weight	0 67(208)	0 85(208)	0 40(114)	0 43(114)	0.32(161)	0 41(118)	0.31 (97)	0 09(178)	0 40(171)	0.31(149)	0 38(161)
Height	0 55(476) 0 75(107)		0 42(114) 0 53 (46)	0.55(114) 0 76 (46)	0 25(349) 0 25 (76)	0.29(349)	0.30(229) 0 45 (56)	0 15(376) 0 16 (95)	0.38(374) 0.28 (94)	0.23(345) 0 19 (87)	0 30(352) 0 34 (88)
Tibia length	0 52(208) 0.58(104)	0 88(208) 0 78(104)	0.35(114) 0 46 (46)	0 48(114) 0 59 (46)	0 26(161) 0.22 (76)	0 37(118)	0 16 (97) 0.34 (56)	0 13(178) 0 27 (95)	0.39(171) 0.39 (95)	0 27(149) 0 16 (89)	0 40(161) 0 28 (88)
Radius length	0.50(208) 0.57(104)	0 76(208) 0 62(104)	0.33(114) 0 55 (46)	0 37(114) 0 58 (46)	0 20(161) 0 20 (76)	0.24(118)	0 28 (97) 0 22 (56)	0 07(178) 0 22 (95)	0.34(171) 0.33 (95)	0 28(155) 0 09 (89)	0 30(161) 0 10 (88)
Condylar breadth	0 0 (208) 0 84(104)	0 60(208) 0.52(104)	0.34(114) 0 49 (46)	0 24(114) 0 47 (46)	0.33(161) 0.28 (76)	0.38(118)	0.32 (97) 0 22 (56)	0 09(178) 0 13 (94)	0.30(171) 0 11 (93)	0 25(149) 0 16 (90)	0 30(161) 0,27 (89)
Bistyloid breadth	0 45(208)	0 40(208)	0 20(114)	0 17(114)	0 24(161)	0.36(118)	0 20 (97)	—0 02(178)	0.29(171)	0 17(149)	0 20(161)
Handgrip	0 51(208) 0.57(103)	0.27(208) 0 52(102)	0.25(114) 0 18 (39)	0 11(114) 0 20 (39)	0.22(161) 0 19 (75)	0 18(112)	0 05 (97) 0.29 (53)	0 00(178) 0 03 (94)	0 15(171) 0 03 (93)	0 11(149) 0 00 (89)	0 13(161) 0 10 (89)
Shoulder thrust	0 41(206) 0 46(100)	0 15(206) 0.38(100)	0 23(113) 0.23 (35)	0 10(113) 0 19 (35)	0 16(160) 0 15 (75)	0 10(111)	0 02 (97) 0 02 (55)	0 03(176) —0 03 (86)	0 17(169) —0 03 (86)	0 13(147) 0 00 (85)	0 05(159) —0 03 (81)
Shoulder pull	0 19(206) 0 55(100)	0 20(206) 0 15(100)	0 18(113) 0 18 (35)	0 12(113) 0 31 (35)	0 17(160) 0 17 (71)	0.20(111)	0 06 (97) 0 10 (52)	0 09(176) —0 02 (89)	0 19(169) 0 00 (88)	0 06(147) 0 06 (85)	0 08(159) 0 10 (84)
Skinfold back	0 11(208) 0 10(103)	—0 07(208) 0 10(103)	0.32(114) 0 18 (46)	0 08(114) 0.38 (46)	0 00(161) —0 18 (75)	0 03(112)	—0 05 (97) —0 12 (55)	0.37(178) 0 11 (92)	—0 02(171) —0 16 (91)	—0 01(149) 0 12 (88)	—0 00(161) 0 17 (87)
Skinfold lat.chest	0 15(208) 0.35(103)	—0 09(208) 0 13(103)	0 27(114) 0 55 (46)	—0 01(114) 0 41 (46)	0 01(161) —0 08 (75)	0 06(118)	—0 16 (97) —0 10 (55)	0 32(178) 0 08 (92)	—0 05(171) —0 14 (91)	0 02(149) 0 10 (88)	—0 01(161) 0 12 (87)
Skinfold abdomen	0 13(208) 0 16(103)	0 01(208) 0.22(103)	0 23(114) 0.50 (46)	0 02(114) 0 47 (46)	—0 01(161) —0 07 (75)	—0 06(118)	—0 12 (97) —0 02 (55)	0.36(178) 0 17 (92)	0 02(171) 0 00 (91)	0 01(149) 0 13 (88)	0 03(161) 0 21 (87)
Weight minus fat free weight	[illegible]	0 21(208)	0.27(114)	0 01(114)	0 02(161)	—0 01(118)	—0 10 (97)	0 1 (178)	—0 00(171)	0 01(149)	—0 10(161)
Circumf chest	0 85(118)	0.37(118)	0.33 (57)	0 10 (57)	0.30(111)	0.30 (81)	0 10 (43)	0 18(121)	0 17(120)	0 0 (119)	0 02(117)

	Weight	Height	Weight at 13	Height at 13	Birth weight	Birth length	Birth weight of sibs	Mother' weight	Mother' height	Father' weight	Father' height
Shoe size	0.61([illegible])	0.73([illegible])	0.18([illegible])	0.34([illegible])	0.30([illegible])	0.33([illegible])	0.15([illegible])	0.11([illegible])	0.26([illegible])	0.24([illegible])	0.36([illegible])
Weight at 13	0.55([illegible]) 0.58([illegible])	0.42([illegible]) 0.53([illegible])		0.73([illegible]) 0.80([illegible])	0.35([illegible]) 0.19([illegible])	0.31([illegible])	0.23([illegible]) 0.10([illegible])	0.18([illegible]) 0.11([illegible])	0.26([illegible]) 0.01([illegible])	0.17([illegible]) 0.20([illegible])	0.01([illegible]) 0.18([illegible])
Height at 13	0.36([illegible]) 0.65([illegible])	0.55([illegible]) 0.78([illegible])	0.72([illegible]) 0.80([illegible])		0.32([illegible]) 0.16([illegible])	0.41([illegible])	0.30([illegible]) 0.26([illegible])	0.14([illegible]) 0.13([illegible])	0.31([illegible]) 0.30([illegible])	0.11([illegible]) 0.20([illegible])	0.02([illegible]) 0.30([illegible])
Birth weight	0.29([illegible]) 0.33([illegible])	0.25([illegible]) 0.25([illegible])	0.35([illegible]) 0.19([illegible])	0.32([illegible]) 0.16([illegible])		0.79([illegible])	0.52([illegible]) 0.52([illegible])	0.13([illegible]) 0.16([illegible])	0.17([illegible]) 0.19([illegible])	−0.06([illegible]) 0.07([illegible])	−0.04([illegible]) 0.02([illegible])
Birth length	0.33([illegible])	0.29([illegible])	0.31([illegible])	0.34([illegible])	0.79([illegible])		0.37([illegible])	0.21([illegible])	0.21([illegible])	0.05([illegible])	0.03([illegible])
Sibs' birth weight	0.29([illegible]) 0.21([illegible])	0.30([illegible]) 0.45([illegible])	0.23([illegible])	0.30([illegible])	0.52([illegible]) 0.52([illegible])	0.37([illegible])		0.15([illegible]) 0.32([illegible])	0.26([illegible]) 0.21([illegible])	0.00([illegible]) 0.00([illegible])	0.03([illegible]) 0.21([illegible])
Mother weight	0.17([illegible]) 0.19([illegible])	0.15([illegible]) 0.16([illegible])	0.18([illegible]) 0.11([illegible])	0.11([illegible]) 0.13([illegible])	0.13([illegible]) 0.16([illegible])	0.31([illegible])	0.15([illegible]) 0.32([illegible])		0.30([illegible]) 0.38([illegible])	0.07([illegible]) 0.06([illegible])	0.07([illegible]) 0.16([illegible])
Mother' height	0.27([illegible]) 0.08([illegible])	0.30([illegible]) 0.28([illegible])	0.26([illegible]) 0.01([illegible])	0.31([illegible]) 0.20([illegible])	0.17([illegible]) 0.19([illegible])	0.24([illegible])	0.25([illegible]) 0.21([illegible])	0.33([illegible]) 0.36([illegible])		0.22([illegible]) −0.01([illegible])	0.22([illegible]) 0.11([illegible])
Father' weight	0.22([illegible]) 0.30([illegible])	0.22([illegible]) 0.17([illegible])	0.17([illegible]) 0.21([illegible])	0.11([illegible]) 0.20([illegible])	−0.08([illegible]) 0.07([illegible])	0.05([illegible])	0.00([illegible]) 0.00([illegible])	0.07([illegible]) 0.06([illegible])	0.22([illegible]) −0.01([illegible])		0.16([illegible]) 0.47([illegible])
Father' height	0.17([illegible]) 0.38([illegible])	0.30([illegible]) 0.34([illegible])	0.01([illegible]) 0.18([illegible])	0.02([illegible]) 0.30([illegible])	−0.04([illegible]) 0.23([illegible])	0.03([illegible])	0.03([illegible]) 0.21([illegible])	0.07([illegible]) 0.16([illegible])	0.22([illegible]) 0.11([illegible])	0.40([illegible]) 0.47([illegible])	

For evaluation of significance, See Appendix V

Appendix III

Table 2. Correlation coefficients of intelligence tests.
(Upper rows — Series B. Lower rows — Series A)

		Subtest A	B	C	D	Mean score
	n	465	465	465	465	465 95
Weight	467 109	0.14	0.14	0.11	0.09	0.14 0.02
Height	467 107	0.19	0.17	0.18	0.19	0.22 0.07
Tibia length	208 104	0.04	0.03	0.07	0.00	0.01 —0.00
Radius length	208 104	0.01	0.01	0.04	0.01	0.04 —0.04
Condylar breadth	208 104	—0.00	—0.03	—0.08	—0.02	—0.03 0.03
Bistyloid breadth	208	—0.13	—0.20	—0.20	—0.03	—0.16
Handgrip	208 102	0.04	0.01	0.01	0.16	0.04 0.10
Shoulder thrust	206 100	—0.03	—0.05	0.00	0.13	—0.00 0.00
Shoulder pull	206 100	0.01	0.01	0.03	0.07	0.03 —0.02
Skinfold back	208 103	0.10	0.08	0.09	—0.06	0.06 0.11
Skinfold lat. chest	206 103	0.01	—0.01	0.02	—0.11	—0.03 0.12
Skinfold abdomen	208 103	0.09	0.01	0.03	—0.13	0.00 0.16
Weight at 13	114 40	—0.08	—0.01	0.03	—0.05	—0.01 0.11
Height at 13	114 40	0.02	0.04	0.13	—0.01	0.05 0.03
Birthweight	369 6	0.08	0.10	0.02	0.08	0.08 0.01
Mother's weight	376 95	—0.01	—0.06	—0.01	—0.0[illegible]	—0.05 —0.13
Mother's height	374 94	0.02	0.01	0.00	0.01	0.01 —0.01
Father's weight	345 91	0.03	0.02	0.02	—0.00	0.01 0.02
Father's height	352 90	0.11	0.07	0.01	0.08	0.06 0.01

Table 2. (Cont).

		Subtest A	B	C	D	Mean score
		465	465	465	465	465 98
Manual labour	207 107	—0.30□	—0.31□	—0.27□	—0.10□	—0.29□ —0.32□
Intellectual work	207 107	0.40□	0.45□	0.35□	0.23□	0.42□ 0.41□
School-education	412 108	0.62□	0.59□	0.39□	0.38□	0.60□ 0.56□
Socio-economic level	202 108	0.30□	0.24□	0.16□	0.22□	0.28□ 0.36□
Urbanization	203 106	0.14□	0.16□	0.22□	0.00□	0.15□ 0.09□
Number of Father's sibs	374 96	—0.04	—0.03	—0.03	—0.02	—0.04 0.00
Paternal cousins	359 95	0.01	—0.00	—0.06	—0.04	—.005 0.00
Mother's sibs	384 98	—0.25	—0.24	—0.10	—0.10	—0.20 —0.09
Maternal cousins	363 97	—0.20	—0.18	—0.09	—0.10	—0.17 —0.01
Sibs	398 98	—0.19	—0.17	—0.19	—0.11	—0.20 —0.21
Mother's age at menarche	356 98	—0.13	—0.15	—0.15	—0.15	—0.16 0.05
Mother's age at 1st parturition	374	0.1	0.12	0.12	0.07	0.14

For evaluation of significance, See Appendix V

Appendix III

Table 3 Length factor correlation coefficients.
(Upper rows Series B — lower rows Series A)

	n	Height	Tibia	Radius	Shoe size
		467 107	208 104	208 104	204 —
Height	46[illegible] 107		0.88 0.78	0.[illegible]6 0.62	0.[illegible]3
Tibia length	208 104	0.88 0.78		0.74 0.68	0.63
Radius length	208 104	0.76 0.62	0.74 0.68		0.64
Shoe size	204 —	0.73	0.63	0.64	
Weight	467 107	0.58 0.74	0.52 0.58	0.50 0.57	0.64
Fat free weight	208 —	0.85	0.75	0.72	0.[illegible]8
Condylar breadth	208 104	0.60 0.52	0.51 0.38	0.51 0.40	0.64
Bistyloid breadth	208 —	0.40	0.30	0.43	0.49
Circumf. chest	146 —	0.37	0.36	0.33	0.44
Handgrip	208 102	0.27 0.52	0.23 0.34	0.24 0.41	0.3[illegible]
Shoulder thrust	206 100	*0.15* 0.38	*0.11* 0.16	*0.22* 0.30	*0.26*
Shoulder pull	206 100	0.20 0.45	0.17 0.31	0.22 0.37	0.26
Skinfold back	208 103	—0.07 0.10	—0.00 0.10	—0.17 0.0[illegible]	0.00
Skinfold lat. chest	208 103	—0.00 0.13	—0.09 0.14	—0.14 0.04	0.01
Skinfold abdomen	208 103	0.00 0.22	—0.02 0.22	—0.14 0.12	0.06
Weight minus fat-free weight	208 —	—0.21	—0.21	—0.20	—0.06
Weight at 13	114 40	0.42 0.53	0.35 0.46	0.33 0.55	0.48
Height at 13	114 40	0.55 0.[illegible]8	0.48 0.59	0.37 0.58	0.31
Birthweight	(161)[1] 349 [illegible]8	0.25 0.25	0.26 0.22	0.20 0.19	0.30
Birthlength	(112)[1] 249 —	0.29	0.37	0.21	0.33

Table 3. (Cont.)

	n	Height	Tibia	Radius	Shoe size
		467	208	208	204
		107	104	104	—
Mother' weight	(172)[1]				
	376	0.15	0.13	0.07	0.11
	93	0.16	0.27	0.22	
Mother' height	(171)[1]				
	371	0.38	0.39	0.34	0.25
	94	0.38	0.39	0.33	
Father weight	(159)[1]				
	345	0.33	0.37	0.28	0.35
	91	0.17	0.16	0.08	
Father' height	(161)[1]				
	352	0.39	0.40	0.30	0.36
	90	0.34	0.28	0.19	
Blood pressure systolic[4]	208	0.22	0.20	0.26	0.23
	101	—0.06	0.00	0.11	
Blood-pressure diastolic[4]	206	0.10	0.11	0.13	0.12
	104	—0.21	—0.09	—0.03	
Mean score intel. test	485□	0.22□	0.04□	0.04□	0.05□
	96□	0.07□	0.00□	—0.04□	
School-education	412□	0.20□	—0.02□	—[illegible]□	0.07□
	106□	0.05□	0.03□	0.09□	

Pair of observations of series B as to tibia, radius and shoe-size.

Difference between series A and B may be due to an increase of the blood pressure but also t retardation of growth with longer duration of diabetes.

For evaluation of significance See Appendix V

Appendix III

Table 3. *Length factor correlation coefficients.*
(Upper rows Series B — lower rows Series A)

	n	*Height*	*Tibia*	*Radius*	*Shoe size*
	467 107		208 104	208 104	204 —
Height	467 107		0.88 0.78	0. 6 0.62	0.73
Tibia length	208 104	0.88 0.78		0. 4 0.68	0.63
Radius length	208 104	0.76 0.62	0.74 0.68		0.64
Shoe size	204 —	0.73	0.63	0.64	
Weight	467 107	0.58 0.74	0.52 0.58	0.50 0.57	0.64
Fat free weight	208 —	0.85	0.75	0.72	0.76
Condylar breadth	*208* 104	*0.60* 0.52	*0.51* 0.38	*0.51* 0.40	*0.64*
Bistyloid breadth	208 —	0.40	0.39	0.43	0.49
Circumf. chest	146 —	0.37	0.36	0.33	0.44
Handgrip	208 102	0.27 0.52	0.23 0.34	0.24 0.41	0.37
Shoulder thrust	206 100	0.15 0.38	0.11 0.16	0.22 0.30	0.26
Shoulder pull	206 100	0.20 0.4	0.17 0.31	0.22 0.37	0.26
Skinfold back	208 103	—0.07 0.10	—0.09 0.10	—0.17 0.07	0.00
Skinfold lat. chest	208 103	—0.09 0.13	—0.09 0.14	—0.14 0.04	0.01
Skinfold abdomen	208 103	0.00 0.22	—0.02 0.22	—0.14 0.12	0.06
Weight minus fat-free weight	208 —	—0.24	—0.21	—0.20	—0.06
Weight at 13	114 40	0.42 0.53	0.35 0.46	0.33 0.55	0.48
H ight at 13	114 40	0.65 0. 6	0.48 0.59	0.37 0.58	0.31
Birthweight	(161)[1] 349 8	0.25 0.25	0.26 0.22	0.20 0.19	0.30
Birthlength	(112)[1] 240 —	0.29	0.37	0.24	0.33

Table 4 (Cont.)

		Condylar breadth	Bistyloid breadth	Circumf chest
		208 104	208 —	146 —
Father's weight	159 91	0.25 0.16	0.17	0.07
Father's height	161 90	0.30 0.26	0.20	0.02
Blood pressure systolic	208 104	0.18 —0.01	0.18	0.07
Blood-pressure diastolic	208 104	0.06 —0.08	0.12	0.01
Mean score intel. test	465 96	—0.03□ —0.01□	—0.16□	0.11□
Manual labour	207 107	0.14□ 0.18□	0.26□	0.16□
Intellectual work	207 107	—0.07□ —0.11□	—0.28□	0.02□
School-education	412 103	0.01□ —0.04□	—0.20□	0.00□
Urbanization	203 106	—0.04□ —0.11□	—0.25□	0.08□

For evaluation of significance, see Appendix A

Appendix III

Table 4 Sturdiness factor correlation coefficients.
(Upper rows series B — lower rows series A)

	n	Condylar breadth	Bistyloid breadth	Circumf chest
n		208 104	208 —	146 —
Condylar breadth	208 104		0 61	0 42
Bistyloid breadth	208 —	0 61		0.36
Circumf. chest	146 —	0 42	0.36	
Weight	467 107	0 47 0.57	0 65	0 45
Fat-free weight	208 —	0.82	0 5	0.43
Height	467 107	0 60 0.52	0 40	0.37
Tibia length	208 104	0 51 0.38	0.39	0.36
Radius length	208 104	0.54 0 40	0 43	0.33
Handgrip	208 102	0 41 0.52	0.43	0.48
Shoulder thrust	206 100	0.32 0.39	0.36	0.45
Shoulder pull	206 100	0.31 0 40	0.31	0.51
Skinfold back	208 103	0 13 0 13	—0.12	0.33
Skinfold lat. chest	208 103	0.12 0.21	—0 11	0.31
Skinfold abdomen	208 103	0.14 0.27	—0.10	0.21
Weight minus fat free weight	208 —	—0 09	—0.24	0.33
Weight at 13	114 40	0.34 0 48	0.20	0.33
Height at 13	114 40	0.24 0.47	0.17	0.19
Birthweight	161 70	0.33 0.28	0.21	0.36
Birthlength	112 —	0.38	0.36	0.30
Mother's weight	1[illegible]2 85	0.09 0.13	—0 02	0.18
Mother's h[illegible]ight	1[illegible]1 84	0.30 0.10	0.20	0.17

Table 5. (Cont.)

		Handgrip	Shoulder thrust	Shoulder pull
		208 102	208 100	206 100
Father's weight	157 91	0.11 0.03	0.13 0.03	0.06 0.03
Father's height	159 90	0.13 0.05	0.06 0.01	0.08 0.18
Blood-pressure systolic	208 104	0.15 0.08	0.08 −0.18	0.10 0.09
Blood-pressure diastolic	206 104	0.07 −0.04	0.11 −0.17	0.05 −0.02
Manual labour	207□ 107□	0.29□ 0.29□	0.30□ 0.28□	0.19□ 0.27□
Intellectual work	207□ 107□	0.11□ 0.03□	−0.19□ 0.11□	−0.10□ 0.14□
School-education	412□ 108□	−0.07□ −0.11□	−0.06□ −0.15□	−0.03□ −0.08□
Urbanization	203□ 106□	−0.07□ −0.17□	−0.06□ −0.15□	−0.01□ −0.03□

For evaluation of significance see Appendix V

Appendix III

Table 5 Muscle factors correlation coefficients.
(Upper rows series B — lower rows series A)

	n	Handgrip	Shoulder thrust	Shoulder pull
n		203 102	206 100	206 100
Handgrip	203 102		0.73 0.64	0.66 0.58
Shoulder thrust	206 100	0.73 0.61		0.67 0.62
Shoulder pull	206 100	0.66 0.58	0.67 0.62	
Weight	467 107	0.51 0.57	0.44 0.46	0.49 0.53
Fat free weight	208 —	0.41	0.31	0.31
Height	467 107	0.27 0.52	0.15 0.38	0.20 0.45
Tibia length	208 104	0.23 0.34	0.11 0.16	0.17 0.30
Radius length	203 104	0.34 0.45	0.22 0.31	0.22 0.37
Condylar breadth	208 104	0.41 0.52	0.32 0.39	0.31 0.40
Bistyloid breadth	208 —	0.43	0.36	0.31
Circumf. chest	146 —	0.48	0.45	0.51
Skinfold back	203 103	0.05 0.09	0.05 0.01	0.12 0.15
Skinfold lat. chest	208 103	0.06 0.02	0.06 —0.03	0.12 0.04
Skinfold abdomen	208 103	0.02 0.05	0.00 0.05	0.10 0.04
Weight minus fat free weight	208 —	0.18	0.21	0.27
Weight at 13	114 40	0.25 0.18	0.23 0.23	0.18 0.18
Height at 13	114 40	0.11 0.26	0.10 0.19	0.17 0.31
Birthweight	180 78	0.22 0.18	0.15 0.14	0.12 0.17
Birthlength	111 —	0.18	0.10	0.20
Mother's weight	170 95	0.09 0.00	0.08 —0.03	0.09 —0.02
Mother's height	169 91	0.15 0.10	0.17 0.00	0.19 0.00

Table 8 (Cont.)

		Handgrip	Shoulder thrust	Shoulder pull
		208 102	208 100	206 100
Father's weight	187 91	0.11 0.03	0.13 0.03	0.04 0.03
Father's height	159 90	0.13 0.05	0.05 0.01	0.08 0.18
Blood-pressure systolic	208 101	0.15 0.06	0.08 —0.15	0.10 0.09
Blood-pressure diastolic	208 104	0.07 —0.04	0.11 —0.17	0.03 —0.02
Manual labour	207□ 107□	0.29□ 0.29□	0.30□ 0.28□	0.19□ 0.27□
Intellectual work	207□ 107□	0.11□ 0.03□	—0.19□ 0.11□	—0.10□ 0.14□
School-education	412□ 106□	—0.07□ —0.11□	—0.08□ —0.15□	—0.03□ —0.08□
Urbanization	203□ 106□	—0.07□ —0.17□	—0.06□ —0.15□	—0.03□ —0.03□

For evaluation of significance, See Appendix V

Appendix III

Table 5 Muscle factors correlation coefficients.
(Upper rows series B — lower rows series A)

	n	Handgrip	Shoulder thrust	Shoulder pull
n		208 102	206 100	206 100
Handgrip	208 102		0 73 0.61	0.66 0.58
Shoulder thrust	206 100	0.73 0 64		0.67 0 62
Shoulder pull	206 100	0 66 0 58	0.67 0 62	
Weight	467 107	0.61 0.57	0 44 0.46	0.49 0.55
Fat free weight	208 —	0 41	0.31	0.31
Height	467 107	0.27 0.52	0.15 0.38	0.20 0.45
Tibia length	206 104	0.23 0.34	0.11 0 16	0.17 0.30
Radius length	208 104	0.34 0.45	0.22 0.31	0.22 0.37
Condylar breadth	208 104	0 41 0.52	0.32 0.39	0.31 0.40
Bistyloid breadth	208 —	0.43	0.36	0.31
Circumf. chest	146 —	0.48	0.45	0.51
Skinfold back	208 103	0 05 0.09	0.05 0.01	0.12 0.15
Skinfold lat. chest	208 103	0.06 0.02	0.06 —0.03	0 12 0.04
Skinfold abdomen	208 103	0.02 0.05	0 00 0.05	0.10 0.04
Weight minus fat free weight	208 —	0 18	0.21	0.27
Weight at 13	114 40	0.25 0.18	0.23 0.23	0.18 0.18
Height at 13	114 40	0.11 0.26	0.10 0.19	0.17 0.31
Birthweight	160 76	0.22 0 18	0.15 0.14	0.12 0.17
Birthlength	111 —	0 18	0.10	0.20
M ther s weight	170 95	0.09 0.00	0.03 —0.03	0.09 —0.02
Mother' height	169 94	0.15 0.10	0.17 0.00	0.19 0.00

Table 6 (Cont.)

		Skinfold back	Skinfold lat. chest	Skinfold abd.	Weight minus fat-free weight
		206 103	208 103	206 103	206 —
Mother's height	171 91	−0.02 −0.16	−0.05 −0.14	0.02 0.00	−0.04
Father's weight	159 91	−0.04 0.12	0.02 0.10	0.04 0.13	0.01
Father's height	161 90	−0.08 0.17	−0.02 0.12	0.03 0.21	−0.16
Blood-pressure systolic	208 101	0.05 0.27	0.04 0.16	0.04 0.15	0.06
Blood-pressure diastolic	208 101	0.05 0.16	0.07 0.05	0.02 0.03	0.05
Hairgrowth chest	208 103	0.23□ 0.24□	0.21□ 0.14□	0.17□ 0.20□	0.22□
Manual labour	207 107	−0.17□ 0.00□	−0.12□ −0.16□	−0.20□ −0.07□	−0.03□
Intellectual work	207 107	0.15□ 0.13□	0.09□ 0.11□	0.18□ 0.10□	0.07□
School-education	412 104	0.12□ −0.02□	0.04□ 0.15□	0.14□ 0.06□	0.05□
Urbanization	203 106	0.22□ −0.21□	0.16□ 0.03□	0.16□ 0.00□	0.11□

Judged by [illegible] five-grade continuous scale according to LINDEGÅRD (1956).

For evaluation of significance see Appendix I

Appendix III

Table 6. *Fat factor correlation coefficients.*

(Upper rows Series B — lower rows Series A)

	n	Skinfold back	Skinfold lat. chest	Skinf ld abdomen	Weight minus fat free weight
		208 103	208 103	208 103	208 —
Skinfold back	208 103		0 86 0 71	0.84 0.73	0.60
Skinfold lat. chest	208 103	0 86 0.71		0.81 0.85	0.66
Skinfold abdomen	208 103	0.84 0 73	0 84 0 85		0.57
Weight minus fat free weight	208 —	0.60	0 66	0 57	
Weight	467 107	0 41 0.40	0.45 0.35	0.43 0.46	0 49
Fat free weight	208 —	—0 05	—0.05	—0 00	—0.28
Height	467 107	—0 07 0.10	—0 09 0 13	0 00 0.22	—0.21
Tibia length	208 101	—0.09 0 10	—0.09 0 14	—0 02 0.22	—0.21
Radius length	208 104	—0.17 0 07	—0.14 0 04	—0 14 0.12	—0.20
Condylar breadth	*208* 104	*0.13* 0.13	*0 12* 0.21	*0.14* 0.2	*—0.09*
Bistyloid breadth	208 —	—0.12	—0 11	—0 10	—0.29
Circumf chest	146 —	0.33	0.31	0.21	0.33
Handgrip	208 *102*	0.05 0.08	0.06 —0 02	0.02 0 01	0 18
Shoulder thru t	206 100	0.05 0 01	0.06 —0.02	0 00 —0.05	0.21
Shoulder pull	206 100	0.12 0 15	0.12 0.01	*0 10* 0.01	0.27
Weight at 13	114 40	0.32 0.48	0.27 0.55	0.23 0.56	0.27
Height at 13	114 40	0.08 0.38	—0.01 0 44	0.02 0.47	—0.02
Birthweight	161 76	0 09 —0.18	0.01 —0.05	0.03 *—0 07*	0 02
Birthl ngth	112 —	0.03	0.06	—0.06	—0 01
M ther' weight	*172* 95	*0.37* 0 11	0.32 0 08	0.36 0.16	0.17

Appendix IV

Table 1 In the calculation f the risk of person with diabetic relative being diabetes gene carrier ith the possibility of developing diabetes the following expressions were used (largely according to *Hultkrantz & Dahlberg*, 1927).
The proportion of the assumed diabetes inducing gene in the population is p and of corresponding normal allele q, where $p+q=1$

Inheritance / Diabetic [illegible]	Recessiv	Dominant
Sib	$1/4\,(1+p)^2$	$1-\frac{q^2(3+q)}{4(1+q)}$
Parent or child	p	$1-\frac{q^2}{1+q}$
Grandparent, uncle or aunt	$1/2\,p\,(1+p)$	$1-\frac{q^2(2+q)}{2(1+q)}$
First cousin	$1/4\,p\,(1+3p)$	$1-\frac{q^2(4+3q)}{4(1+q)}$

Appendix III

Table — Coefficients of correlation between blood pressure and various somatic data. (Upper rows series B — lower rows series A)

	n	Syst	Diast
		208 104	208 104
Systolic pressure	208 104		0.60 0.69
Diastolic pressure	208 104	0.60 0.69	
Weight	467 107	0.21 0.19	0.11 0.02
Fat-free weight	208	0.27	0.14
Height	467 107	0.22 —0.05	0.10 —0.21
Tibia length	208 104	0.20 0.00	0.11 —0.09
Radius length	208 104	0.26 0.11	0.13 —0.03
Condylar breadth	208 104	0.18 —0.04	0.06 —0.06
Distyloidbreadth	208	0.18	0.12
Circumf. chest	146	0.07	0.01
Handgrip	208 102	0.15 0.06	0.07 —0.01
Shoulder thrust	207 100	0.08 —0.15	0.11 —0.17
Shoulder pull	208 100	0.10 —0.11	0.05 —0.09
Skinfold back	208 103	—0.02 0.27	—0.02 0.16
Skinfold lat. chest	208 103	0.00 0.16	—0.01 0.05
Skinfold abdomen	208 103	0.06 0.15	0.02 0.03
Weight minus fat free weight	208	—0.03 0.46	—0.03 0.24

Appendix IV

Table 2. Risk of being a gene carrier able to develop a disease on assuming different frequencies of a mutant pathogenic allele and recessive or dominant inheritance.

Type of relative \ Gene-frequency	Recessive inheritance							Dominant inheritance				
	1/2	1/4	1/5	1/6	1/7	1/8	1/∞	1/2	1/5	1/10	1/100	1/∞
Sib	0.56	0.39	0.36	0.31	0.33	0.32	0.25	0.85	0.66	0.59	0.51	0.50
Parent or child	0.50	0.25	0.20	0.17	0.14	0.13	0	0.83	0.64	0.57	0.51	0.50
Grandparent, uncle or aunt	0.38	0.16	0.12	0.10	0.08	0.07	0	0.70	0.50	0.38	0.20	0.25
First cousin	0.31	0.11	0.08	0.06	0.05	0.01	0	0.77	0.43	0.20	0.11	0.13
No relative	0.25	0.06	0.01	0.03	0.02	0.02	0	0.75	0.36	0.19	0.02	0

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ACTA MEDICA SCANDINAVICA

SUPPLEMENTUM 374

VITAMIN B_{12} DEFICIENCY IN FISH TAPEWORM CARRIERS

A clinical and laboratory study

BY

ILMARI PALVA



ACCOMPANIES VOL. 171

HELSINKI 1962

From the Medical Department and the Laboratory Department
Central Hospital of Northern Karelia, and
Second Medical Department, University of Helsinki

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CONTENTS

CONTENTS

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Helsinki, December 1961

I P

Fresh meat fattens,
fresh fish kills.

Finnish proverb

A SURVEY OF PUBLICATIONS ON TAPEWORM PERNICIOUS ANAEMIA

Early observations on the relationship of the fish tapeworm to pernicious anaemia

Prior to 1885, Albrecht in St Petersburg drew attention to the finding of fish tapeworm in certain cases of pernicious anaemia on necropsy.

The first clinical notes of tapeworm anaemia appeared in 1885, Hoffman and Botkin observed infestation by fish tapeworm in many of their cases of pernicious anaemia. The anaemia was relieved after expulsion of the parasite. In 1886, there appeared more detailed studies by Reyher and Runeberg on the beneficial effect of worm cure in some cases of pernicious anaemia. During the years that followed, reports were published concerning a number of patients with fish tapeworm and pernicious anaemia — by Mitzkumer (1886), Lichtheim (1887), Schapiro (1888), Müller (1889), Wyjarjukki (1889), Dehio (1891), Litten (1892), Vlayeff (1894) and others. These authors believed that the tapeworm might have caused pernicious anaemia in their patients.

Several investigators questioned the validity of these statements; most of them had never seen a case of tapeworm anaemia. In a thesis presented in 1894, Schauman gave conclusive evidence that the fish tapeworm might cause pernicious anaemia. Following an expulsion of the worm, 60 of his 72 patients recovered, and 12 died before or shortly after this treatment.

The incidence of tapeworm pernicious anaemia among carriers of fish tapeworm

Ehrström (1928) based his calculations of the incidence of tapeworm anaemia on the results of a questionnaire sent to members of the medical profession in Finland. He estimated that one carrier in 5,000 to 10,000 develops tapeworm anaemia. In a series of military patients, Seppä (1927) found one case of tapeworm anaemia among 659 tapeworm carriers. During the war Totterman (1944) observed the occurrence of tapeworm and megaloblastic tapeworm anaemia in the patients of an emergency hospital and its out-patient department. He assumed the frequency of tapeworm carriers among his civilian patients to hold good for the whole population of the hospital district. Working on the premise that most of the tapeworm pernicious anaemia cases were treated in the hospital, in 1942 he calculated that among the 113 soldiers who were tapeworm carriers there was one with megaloblastic anaemia the cor

peripheral blood of pernicious anaemia patients. Zadek (1921) reported megaloblastic erythropoiesis in the bone marrow of Addisonian pernicious anaemia patients. A number of investigators then stated that megaloblasts were to be found only in cases of pernicious anaemia — Escudero and Varela (1927 1930) Tempka and Braun (1932) Dameshek (1935) Rohr (1935) Roversi and Tanturri (1935) Segerdahl (1935) and Nordenson (1936)

Megaloblastic erythropoiesis in tapeworm pernicious anaemia was reported by Tötterman (1935, 1939) Before this finding, the criteria of tapeworm pernicious anaemia comprised the occurence of hyperchromic anaemia in a tapeworm carrier and the beneficial effect exerted by worm cure on the anaemia.

Tempka and Braun (1932) devoted attention to the presence of giant granulocytes in pernicious anaemia. Dameshek and Valentine (1937) reported that the changes were most distinct in metamyelocytes. Tötterman (1939) reported giant metamyelocytes in the sternal marrow of tapeworm pernicious anaemia patients. Foy et al. (1950) laid emphasis on the presence of the giant metamyelocytes being just as pathognomonic for pernicious anaemia as that of the megaloblasts. Bastrup-Madsen (1952 954, 1956) Jewesbury (1954) Munch-Petersen (1955) Kristensen and Ohlsen (1956) Hansen and Paulsen (1957) Pedersen et al. (1957) Kristensen et al. (1958) Kristensen and Gormsen (1958) Hansen (1960) and Swedberg (1960) commented on the possibility of detecting giant metamyelocytes in the bone marrow before the appearance of the megaloblasts in incipient cases of pernicious anaemia.

Heinivaara and Kalpalnen (1959, 1960) measured the diameters of myeloid and erythroid cells in incipient tapeworm pernicious anaemia without megaloblasts. They found that the changes in the metamyelocytes displayed pronounced features when a comparison was made between megaloblastic anaemia patients and healthy controls. They also reported that the large-sized metamyelocytes predominated in the bone marrow of patients suffering from Addisonian pernicious anaemia and tapeworm pernicious anaemia. Some of the tapeworm carriers in their series had an increased number of large metamyelocytes, whereas others were comparable with the controls. They considered that an increased number of large-sized metamyelocytes should serve as an indication of the possible presence of incipient megaloblastic anaemia.

Neurological disturbance in tapeworm pernicious anaemia

Björkenheim (1951) made a study of the neurological disturbance in tapeworm pernicious anaemia, and found it to be similar to that in Addisonian pernicious anaemia. He also examined some tapeworm carriers without anaemia, and among these cases was able to detect symptoms similar to those observable in pernicious anaemia patients, although the frequency was not so marked.

Gastric disturbance in tapeworm pernicious anaemia

As regards some tapeworm pernicious anaemia patients, Schauman (1894) observed free hydrochloric acid in Ewald's

responding calculation for the following year gave one in 241. As regards the civilian population his figures were one in 136 (1942) and one in 383 (1943). Tötterman pointed out that nutrition in this country was on a lower level in 1942 than in 1943; this was especially true in respect of deficiency in the amount of animal protein in the diet. Lindström (1929) reported seasonal variations in the occurrence of tapeworm anaemia. In the hospitals of Helsinki, more tapeworm anaemia cases were detected during the period from March to August than during the rest of the year. The seasonal variation of Addisonian pernicious anaemia was on similar lines. Setälä (1948, 1949) studied about one half of the population of Kärkölä commune (in Western Finland) in 1945—1946. He gave the incidence of tapeworm as 2.7 per cent among the original population, and 23.2 per cent among the evacuees from Karelia. He found four cases of tapeworm anaemia among his 300 tapeworm carriers (1 : 75). In Northern Karelia in the spring of 1958 Nyberg (1960, 1961) examined the population of some villages of Pielisjärvi rural district. Among 1344 persons, he found tapeworm in 366 (27.2 per cent); eight of them had megaloblastic tapeworm anaemia (1 : 46).

The age distribution of tapeworm pernicious anaemia

The findings of investigators regarding the age distribution of tapeworm anaemia patients have varied somewhat in different series. In the earlier series (Schauman 1894, Schauman and Saltzman 1925) many more young persons were found among the tapeworm anaemia patients than among those suffering from Addisonian pernicious anaemia. In their statistical investigation Tötterman and Ahrenberg (1956) found parallel age distribution curves in tapeworm anaemia and Addisonian pernicious anaemia, although the tapeworm anaemia patients included a slightly larger number of cases affecting young people. The youngest case of tapeworm pernicious anaemia in Finland was observed in a boy aged 9 (Wegelius and Malm 1955). Kisel (1888) reported in St. Petersburg the occurrence of pernicious anaemia and fish tapeworm in a boy aged 6.

The clinical picture of tapeworm pernicious anaemia

Haematological morphology

The peripheral blood count. Schauman (1894) demonstrated that the picture presented by the peripheral blood in tapeworm (pernicious) anaemia was identical with that found in Addisonian pernicious anaemia. In his cases subjected to necropsy, he found also the typical macroscopic picture of pernicious anaemia. Tötterman (1944) studied the Price-Jones curve of the red blood cells in tapeworm pernicious anaemia and found that it bore a similarity to that in Addisonian pernicious anaemia. Telkkä et al. (1954) found no difference in the eosinophil cell counts between tapeworm carriers and controls.

The bone marrow in tapeworm pernicious anaemia. The morphological characteristics of megaloblasts were described by Ehrlich in 1880; some observations were published on their appearance in the

beneficial effect of a worm cure administered to pernicious anaemia patients infested by the fish tapeworm. More detailed studies on the improvement in haemoglobin and red blood cell values in tapeworm anaemia patients after expulsion of the worm were carried out by Runeberg (1886) and Schauman (1894). The reticulocytosis following expulsion of the tapeworm was observed by Belonogova (1928) and confirmed by Saltzman (1929).

Liver p parations

Minot and Murphy (1926, 1927) introduced liver therapy for cases of Addisonian pernicious anaemia. Schottmüller (1928) and Becker (1929, 1930) observed the beneficial effect of parenterally administered raw liver preparations on tapeworm pernicious anaemia before expulsion of the parasite. A reticulocyte crisis after the provision of liver treatment for cases of pernicious tapeworm anaemia was reported by Saltzman (1928), Isaacs et al. (1928), Kerr et al. (1928), Richter et al (1928) and Vesa (1929). Belonogova (1928) observed that the reticulocytosis following liver therapy in tapeworm pernicious anaemia is similar to that following expulsion of the worm, but that it begins somewhat earlier. This was also reported by Saltzman (1929) and v Bonsdorff (1940).

Fol c acid

The reticulocytosis following folic acid treatment of Addisonian pernicious anaemia was noted by Moore et al. (1945).

Bonsdorff (1948) gave folic acid orally to four tapeworm pernicious anaemia patients. They all reacted with reticulocytosis, and an improvement in the blood count. Spies and Stone (1947) stated that neurological disturbance in Addisonian pernicious anaemia did not improve during the course of folic acid treatment. In contrast, some cases developed neurological disturbances while being given folic acid. Björkenheim (1953) noted impairment of the neurological lesion in a patient with tapeworm pernicious anaemia during treatment with folic acid, despite haematological remission.

Vitamin B_{12} preparations

In 1948, Rickes et al., and Smith and Baker succeeded independently in achieving the isolation of vitamin B_{12} from liver. West (1948) found that this preparation, given parenterally in doses of micrograms, was capable of causing haematological remission in Addisonian pernicious anaemia patients.

v Bonsdorff and Gordin (1951) gave vitamin B_{12} and liver preparations orally to eight tapeworm pernicious anaemia patients, and obtained reticulocytosis in respect of five of them.

Int in ic factor prepa at on

Kaipainen and Tötterman (1937) gave daily doses of 1 gram of a crude intrinsic factor preparation from hog pyloric mucosa to tapeworm pernicious anaemia patients, and induced reticulocytosis. By administering daily doses of a grams of the same preparation, more marked reticulocytosis was obtained.

test meal although in the majority of cases no such finding was made. In his cases, also, the proteolytic activity of the stomach was poor. Many investigators confirmed the absence of free hydrochloric acid in tapeworm pernicious anaemia patients when the illness was at its height — Askanazy (1895), Bruhn-Fåhraeus (1896), Rosenqvist (1903), Faber and Lange (1907), Schauman and Levander (1917), Helander (1945), Siurala (1954) and Gräsbeck (1955). In some instances, a return of gastric acidity after remission of tapeworm pernicious anaemia has been observed in several series — Rosenqvist (1903), Schauman and Levander (1917), Hernberg (1947) and Siurala (1956).

Grönberg (1912) studied the gastric secretion of non-anaemic tapeworm carriers. He found that no difference existed in the secretion of hydrochloric acid between tapeworm carriers and healthy persons who served as controls. This result was confirmed by Helander (1945) who further examined the pepsine secretion in tapeworm carriers, and found no disturbance.

Lumme et al. (1954) investigated the uropepsine excretion in tapeworm pernicious anaemia patients. During the height of the disease, they found the uropepsine excretion to be below the normal range in 50 per cent of these cases. In their series, all the Addisonian pernicious anaemia patients showed low values. Among the patients with tapeworm pernicious anaemia, they discovered a slight tendency towards increased uropepsine excretion about one week after a worm cure had been administered.

On necropsy Möller (1897) found atrophic and gastritic changes in the mucosa of the stomach in every case of tapeworm pernicious anaemia which he examined. Wallgren (1923) found chronic inflammation of the gastric mucosa in all his cases of tapeworm pernicious anaemia submitted to necropsy.

In gastroscopic and gastrobioptic studies Siurala (1954) found atrophic superficial gastritis in most cases of tapeworm pernicious anaemia. Twelve months subsequent to a worm cure he made a new examination (Siurala 1956). Many of the patients whose worm cure had proved successful showed improvement of the gastric lesion. In the same group, 19 of the 21 cases had histamine-resistant achlorhydria before the worm cure, and in 10 cases this condition was found 12 months after expulsion of the worm. In five cases, the tapeworm still remained at the time of re-examination. In this group the gastric lesion and achlorhydria had not changed. Siurala also found atrophic gastritis in some tapeworm carriers without anaemia. He discovered no correlation between the gastric lesion and the neurological disturbance in tapeworm pernicious anaemia.

Gräsbeck (1956) studied the vitamin B_{12}-binding principle of the gastric juice and found (1955) a lack of glandular mucoprotein in some cases of tapeworm pernicious anaemia. Five of his 11 cases had histamine-resistant achlorhydria.

Reactions to various forms of treatment in tapeworm pernicious anaemia

Expulsion of the worm

Hoffmann (1885) and Botkin (1885) published the first observations on the

patients with tapeworm pernicious anaemia in remission after worm cures, Tötterman noted a transient fall in the haemoglobin values and the red blood cell counts, with a tendency towards increased colour index. The most effective fraction in the experiments was that containing lysolecitin. Tötterman also stressed the importance of the hypersensitivity in the pathogenesis of tapeworm pernicious anaemia (1944). His opinion (1951) was that the anaemia develops when the tapeworm carrier becomes sensitized to lysolecitin.

Tapeworm pernicious anaemia as a deficiency of vitamin B_{12}

In 1929, Castle presented his theory of the pathogenesis of Addisonian pernicious anaemia. He assumed the presence of two factors. The »extrinsic factor» is a thermostabile dietary one found in food. The »intrinsic factor» is thermolabile, and found in normal human gastric juice. A reaction of the »extrinsic» and the »intrinsic» factors constitutes the »anti-anaemic principle». This is then absorbed in the intestine, and stored in the liver.

By means of summarizing Castle's theory and the results obtained earlier Salteman (1935) concluded that the tapeworm might cause anaemia by inhibiting production of the intrinsic factor, affecting the extrinsic factor, preventing the interaction between extrinsic factor and intrinsic factor, or destroying the anti-anaemic principle.

Hernberg (1936) studied the intrinsic factor activity of the gastric juice in tapeworm pernicious anaemia patients. He gave gastric juice from a patient who had suffered from tapeworm pernicious anaemia six years previously, together with meat, to a patient with Addisonian pernicious anaemia. The latter developed reticulocytosis, and there was an improvement in his blood count. The gastric juice of a patient suffering from tapeworm pernicious anaemia with the disease at its peak also brought about reticulocytosis in an Addisonian pernicious anaemia patient. However, difficulty was experienced in performing this experiment by reason of the small amount of gastric juice obtainable from the tapeworm pernicious anaemia patient. Hernberg further concluded that the gastric secretion in tapeworm pernicious anaemia patients seems to be very deficient, but it is possible that the anti-anaemic factor is not completely lacking in the gastric juice during tapeworm pernicious anaemia. In later experiments of a similar nature Hernberg (1941) found in three out of nine tapeworm pernicious anaemia patients a reticulocytosis following histamine stimulation of the gastric secretion. He established that the amount of gastric juice obtainable by histamine stimulation in tapeworm pernicious anaemia patients during the height of the disease was about a third of the amount obtained from healthy subjects; further to this, he observed a slower improvement in the blood count after worm cure in those cases of tapeworm pernicious anaemia whose gastric secretion was poorest.

In 1939, v. Bonsdorff reported that a liver extract did not lose its anti-anaemic activity as regards Addisonian pernicious anaemia patients when incubated with fish tapeworm. While engaged in a study of the reticulocytosis (1940) in tapeworm

The pathogenesis of tapeworm pernicious anaemia

Constitutional factors

Schauman's investigations concerned with tapeworm pernicious anaemia are summarized in a monograph (Schauman and Saltzman 1925). He gave in this his opinion that a constitutional diathesis is needed for the development of tapeworm pernicious anaemia. The tapeworm is only a precipitating factor. He based his view on the occurrence of many such cases of tapeworm pernicious anaemia who later develop Addisonian pernicious anaemia (Schauman 1910). Reports on cases of this type have also been published by Rosenqvist (1903), Björkenheim (1951) and Kaipainen and Vuorinen (1960). Björkenheim (1951) provided information on some cases of tapeworm pernicious anaemia undergoing a new episode of anaemia with a new tapeworm infestation. The constitutional factor was also stressed by Birkeland (1932) in his monograph in which he reviewed the publications on tapeworm pernicious anaemia up to the year 1930.

Theories of toxic factors in the fish tapeworm

During the end of last century and the first thirty years of this one, the hypothesis was generally accepted that Addisonian pernicious anaemia is caused by toxins (Birkeland 1932). A similar pathogenesis was also proposed for acceptance in respect of tapeworm pernicious anaemia. Botkin (1885) believed that the irritation of the intestine caused by the tapeworm could depress blood formation. Vlayeff (1894) and Willson (1902) were of opinion that in the mucosa of the intestine the tapeworm would interfere with the formation of some important factor needed for blood formation. According to Reyher (1886) some infective element could be transferred to the blood. Schapiro (1888) believed that under certain circumstances the tapeworm might produce a chemical toxin which would bring about destruction of the blood elements. Dehio (1892) offered the explanation that the toxin was liberated from the dead tapeworm or proglottides. The common feature of these hypotheses was the need by the tapeworm of an additional factor in order to produce tapeworm pernicious anaemia. Litten (1892) and v. Noorden (1892) expressed their agreement with this view. The former sought an intermediate factor between the tapeworm and the blood formation.

Many experiments were made concerning the possible toxic agents isolated from the fish tapeworm. Tallqvist (1906, 1907) detected a lipid fraction in fish tapeworm. Given orally this lipid caused a moderate form of haemolytic anaemia in the author himself and hyperchromic anaemia in dogs. Further research on this point was carried out by Seyderhelm (1918) and Becker et al. (1925) with bothriocephalin, and by Nyfeldt (1927) with bothriotoxin. Intravenous tapeworm extracts administered to rabbits caused hyperchromic anaemia in every case. The conclusion drawn by Birkeland (1932) from these experiments was that the fish tapeworm contains toxins which cause anaemia. On giving dried fish tapeworm powder or alcohol extract of the same powder by mouth (1938) or the extract parenterally (1944) to

By the utilization of microbiological methods (*Lactobacillus lactis dorner Lactobacillus leichmannii* and *Euglena gracilis*) Nyberg (1952) investigated the vitamin B_{12} content of the fish tapeworm. The mean amount was 2 3 μg of vitamin B_{12} per gram of dry worm. He found differences between various individual tapeworms but could not establish a difference between the amounts of vitamin B_{12} in worms from tapeworm pernicious anaemia, and those from tapeworm carriers without anaemia.

The binding power of vitamin B_{12} in the tapeworm was examined microbiologically by Kaipainen and Wallén (1954) They incubated a suspension of dried tapeworm with *Escherichia coli 113—3* until the bacteria had used all the vitamin B_{12} available. On subjecting the incubated mixture to heat, there appeared no more free vitamin B_{12} which was detectable with *E. coli 113—3*.

v Bonsdorff and Gordin (1952) carried out clinical investigations on the anti-pernicious activity of the fish tapeworm. They gave dried powdered tapeworm, together with normal gastric juice, to five patients suffering from Addisonian pernicious anaemia. These patients experienced an abatement of the disease. Tapeworm powder alone was ineffective in Addisonian pernicious anaemia. On the other hand, three of their tapeworm pernicious anaemia patients showed improvement in the blood count subsequent to the administration of tapeworm powder alone. v Bonsdorff and Gordin (1953) also found reticulocytosis in one Addisonian pernicious anaemia patient, and in four tapeworm pernicious anaemia patients after intramuscular injections of aqueous extracts from tapeworm. Björkenheim (1957) noted an improvement in the neurological symptoms in Addisonian pernicious anaemia, and in tapeworm pernicious anaemia patients after the injection of tapeworm extracts.

Microbiological determinations of the serum levels of vitamin B_{12} in tapeworm pernicious anaemia patients have been carried out by Nyberg and Östling (1956) Killander (1957) Brante and Ernberg (1958) and Nyberg (1960) All of them found low values, similar to those found in Addisonian pernicious anaemia, in every case of tapeworm pernicious anaemia in their series. Nyberg (1960) studied the serum level of vitamin B_{12} in 358 tapeworm carriers and established decreased values in 51 5 per cent of the cases.

A deficiency in vitamins of the B group other than vitamin B_{12} has also been reported. Markkanen et al. (1960) established lowered urinary excretion of thiamine and pantothenic acid in cases of tapeworm pernicious anaemia. In tapeworm carriers without anaemia, diminished excretions of thiamine were found.

Studies on tapeworm pernicious anaemia using radioactive vitamin B_{12}

Chalet et al. (1950) introduced the vitamin B_{12} labelled with ^{60}Co, which has been extensively used in absorption studies of vitamin B_{12}. The first method utilized was that of determining the radioactivity of the stool after an oral dose of radioactive vitamin B_{12}. Schilling (1953) measured the radioactivity excreted in the urine after an oral dose of radioactive vitamin B_{12} followed by a large parenteral »flushing» dose of non-radioactive vitamin

pernicious anaemia after worm cure, he found (1943) that if patients with tape worm pernicious anaemia maintained a diet which was poor in meat, they did not react with reticulocytosis to the expulsion of the parasite; the addition of meat to their diet caused a reticulocyte reaction. He added dried tapeworm, or aqueous tapeworm extracts (1947) to the mixture of meat and gastric juice. This did not occasion a diminution of the anti anaemic potency of the mixture to the Addisonian pernicious anaemia patients. In addition v. Bonsdorff reported on a series of 11 patients with tapeworm pernicious anaemia (1947). Seven of the cases had been given a mixture of meat and gastric juice. Five of them did not develop reticulocytosis to an extent of more than 3 per cent, two had a reticulocytosis of 12.3 and 20 per cent respectively. In one other instance meat alone caused a reticulocytosis of 11.2 per cent. One patient was administered gastric juice alone, and developed a reticulocytosis of 6 per cent. Another was given a mixture of yeast extract and gastric juice, and reticulocytosis of 8.8 per cent followed. Despite insulin stimulation of the gastric secretion, one patient with tapeworm pernicious anaemia did not develop reticulocytosis to an extent of more than 2 per cent.

v. Bonsdorff and Gordin (1951) gave vitamin B_{12} or liver preparations by the oral route, to eight tapeworm pernicious anaemia patients with the worm in situ. Their report shows that five patients reacted to this treatment with a reticulocytosis of 10 to 22 per cent. In three other cases, the reticulocytosis remained at less than 3 per cent. An addition of gastric juice to the last mentioned three patients did not improve the effect of the oral vitamin B_{12} treatment. The authors believed that the former group had been suffering from a dietary deficiency of vitamin B_{12}. This was then corrected by oral administration of more vitamin B_{12}.

By means of intestinal intubation, v. Bonsdorff (1947) established in cases of tapeworm pernicious anaemia that eggs of *Diphyllobothrium latum* were to be found as high as in the jejunum. In tapeworm carriers without anaemia, the eggs of *Diphyllobothrium latum* were discovered in the ileum, and rather often in the distal part of this section of the intestine. In his discussion of the significance of this, v. Bonsdorff commented: »One must possibly expect that all who suffer from pernicious tapeworm anaemia must have worm high up in the intestines. The results of all my experiments have been in this direction.» To him, it seemed probable that when worm is high up in the intestine it has an opportunity to impair the interaction between the extrinsic and intrinsic factors, and in this way to cause pernicious anaemia.

Vitamin B_{12} in the fish tapeworm and its carriers studied by microbiological methods

Various micro-organisms have been used in the determination of vitamin B_{12}, those most commonly employed being *Lactobacillus leichmannii 313* (Hoffman et al. 1948) *Euglena gracilis var. bacillaris* (Hutner et al. 1948) *Escherichia coli 113—3* (Davis and Mingioli 1950) and *Ochromonas malhamensis* (Ford 1953)

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values amounted to more than 10 per cent. Kaipainen and Ohela (1959) in performing a Schilling II test, gave to ten patients suffering from tapeworm pernicious anaemia an amount of hog intrinsic factor which was double that administered during the course of their investigations in 1957. Prior to the worm cure, they obtained Schilling II test values of 3.0 to 9.5 (mean 8.7) per cent. After expulsion of the worm, the Schilling I test values were 3.0 to 25 (mean 14.6) per cent. In ten patients with tapeworm pernicious anaemia before worm cure, Nyberg (1959, 1960) found Schilling I test values of 0 to 4.9 (mean 1.5) per cent, and Schilling II test values of 0 to 27.8 (mean 4.7) per cent. Subsequent to expulsion of the worm, the Schilling I test values, in six cases, were 7.5 to 25.5 (mean 14.1 per cent). Nyberg interpreted his own results, and those of Kaipainen and Ohela (1957) as indicating that the vitamin B_{12} absorption of the host organism is impaired by the worm, even when the intrinsic factor is present. Kaipainen and Ohela (1957) discussed the significance of the possible disturbance of intrinsic factor activity in tapeworm pernicious anaemia. In their 1959 paper they devoted attention to the combination of the various factors required before a tapeworm carrier develops megaloblastic anaemia. Kaipainen and Ohela further discussed the possibility of tapeworm pernicious anaemia being in the nature of a preliminary phase of Addisonian pernicious anaemia which is present in patients as a latent disease, and owing its emergence to the vitamin B_{12} deficiency caused by the tapeworm.

By means of in vitro experiments, Brante and Ernberg (1957) found that the fish tapeworm is capable of taking up radioactive vitamin B_{12} in a free form, but not when it is bound to hog intrinsic factor. Nyberg (1959) reported that the tapeworm in vitro was capable of taking up a part of the vitamin B_{12} bound to gastric juice, and he concluded that this was probably a matter of the splitting of the vitamin B_{12}-intrinsic factor complex. Kaipainen and Ohela (1959) established that the homogenate of fresh tapeworm, and water extract obtained from dried tapeworm, were capable of breaking up the nondialysable complex of gastric juice and radioactive vitamin B_{12}, and allowing dialysis of a part of the vitamin B_{12}. They assumed that the enzymes present in the worm might result in the disintegration of the complex. In further experiments Nyberg (1960) confirmed that the tapeworm in vitro could take up a part of the radioactive vitamin B_{12} bound to gastric juice or hog intrinsic factor and make dialysable another part of the vitamin. The B_{12} binding capacity of intrinsic factor concentrate and gastric juice was in part lost after incubation with the fish tapeworm. The radioactivity taken up by the worm from the intrinsic factor bound radioactive vitamin B_{12} was found to be largely in nondialysable form in the parasite. In addition, Nyberg established that on incubation of the worm with radioactive vitamin B_{12} without intrinsic factor then in this case the radioactivity was partly removable from the worm by dialysis.

A summary of the various findings reviewed above concerning the investigation of tapeworm pernicious anaemia is that, like Addisonian pernicious anaemia, it is a manifest vitamin B_{12} deficiency

B_{12}. Glass (1954) measured the radioactivity over the liver after an oral dose of *radioactive vitamin* B_{12}. These investigators acquired evidence that Castle's extrinsic factor and anti-anaemic principle, and the vitamin B_{12}, are *identical*.

Nyberg (1957 1958) gave oral doses of radioactive vitamin B_{12} to patients suffering from tapeworm pernicious anaemia. On expulsion of the worm some short time subsequently he was able to establish that in the 16 cases of tapeworm pernicious anaemia the worm had taken 31 to 100 per cent of the dose administered (mean 71 3 per cent) In 126 tapeworm *carriers without anaemia the proportion* of radioactive vitamin B_{12} taken by the worm amounted 4.1 to 8.8 (mean 44.3) per cent The radioactivity was mainly concentrated in the proximal part of the *worm both when calculated as total up*take and as uptake per proglottid or per gram of dry substance. During these experiments Nyberg also took measurements of the radioactivity of the stools. In the *worm and stool taken together he found* 50 7 to 103 per cent of the administered radioactive vitamin B_{12} in the cases of tapeworm pernicious anaemia, and 32 to 94 9 per cent in the tapeworm carriers *without anaemia*. The fish tapeworm did not absorb inorganic radioactive cobalt.

On study of the radioactive vitamin B_{12} taken up by the worm, Nyberg and Gräsbeck (1957) found that no radioactivity was lost when dialysing at 4 C, but that at 37 C the *vitamin* B_{12} was liberated, and also in drying and in boiling the worm. This was mainly due to autolysis.

In the Schilling test Klayman and Brandborg (1955) observed a value of 8.5 per cent in a carrier of fish tapeworm. In 8 tapeworm carriers, Gräsbeck et al (1956) found Schilling test values of 0 to 6 5 per cent (mean 2.4) be*fore worm cure, and 3 3 to 12.8* (mean 8 6) per cent after worm cure. In 50 tapeworm carriers before worm cure, Nyberg et al (1958) found Schilling test values *of 0 to 24.2 per cent (mean 3 9 per cent) During the first week after* expulsion of the worm, 32 tapeworm carriers had Schilling test values of 1 6 to 24.6 (mean 9.3) per cent, and during the following five weeks in 18 carriers in *their series values of 1.4 to 27.4 (mean* 15 0) per cent. Furthermore, in five cases of tapeworm pernicious anaemia Nyberg et al obtained Schilling test values of 0 1 to 2 3 (mean 1 1) per cent before worm *cure and 3 9 to 34.0 (mean 17 2)* per cent one to six weeks later

When the intrinsic factor (from hog pyloric mucosa) is taken as supplement to the Schilling test (Schilling II test) the *values rise in respect of patients* with Addisonian pernicious anaemia (Schilling et *al* 1955) *Kaipainen and Ohela* (1957) on examination of seven patients with tapeworm pernicious anaemia prior to worm cure found Schilling I test values (ordinary) of 0 to 2 0 (mean 1 1) per cent, and *Schilling II test* values (with intrinsic factor) of 3.0 to 7 2 (mean 4 3) per cent After expulsion of the worm the values in the Schilling I test were 6.8 to 16.0 (mean 11 9) per cent. In two cases *of tapeworm pernicious anaemia* Brante and Ernberg (1958) obtained Schilling I test values of 2 and 3 per cent before worm cure. With gastric juice and porcine intrinsic factor *the Schilling II test*

THE SCOPE OF THE PRESENT STUDY

It has become evident that the fish tapeworm is capable of causing megaloblastic anaemia, and other symptoms which are common to Addisonian pernicious anaemia, and intensive studies on the development of this form of anaemia have been engaged in. Recently vitamin B_{12} metabolism has in particular been the subject of considerable research work. The distribution of the available vitamin B_{12} between the tapeworm and the carrier has been examined and the serum levels of vitamin B_{12} in tapeworm carriers determined. Disturbances in the absorption of vitamin B_{12}, and in the serum level of vitamin B_{12}, have been found in most tapeworm carriers, and all those patients suffering from tapeworm pernicious anaemia examined for these factors. The findings provide substantial support for the viewpoint that tapeworm pernicious anaemia is a manifest vitamin B_{12} deficiency brought about by the fish tapeworm.

Nevertheless, the various components of the vitamin B_{12} metabolism in tapeworm carriers have each been investigated in a separate series. The numbers of patients in the series have varied from six to 358 tapeworm carriers, and most of the series have comprised less than ten cases. In some series, methodological inconsistency has been apparent. Our knowledge of the interrelations of the various indications of vitamin B_{12} deficiency in tapeworm carriers is accordingly limited.

The present work has as intention the study of several components of the vitamin B_{12} metabolism in the same group of tapeworm carriers, and in patients with tapeworm pernicious anaemia, with a view to investigating the occurrence and the interrelations of the indications of vitamin B_{12} deficiency obtainable by various methods.

(v. Bonsdorff et al. 1959). Competition between the parasite and the host is waged for the vitamin B_{12} available. On occasion the host then fails to get the quantity of vitamin B_{12} needed for the maintenance of normal erythropoiesis, and develops megaloblastic anaemia. Opinions vary on the importance of various factors in development of the deficiency state. In other carriers of fish tapeworm milder forms of vitamin B_{12} deficiency are present

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THE PRESENT SERIES OF PATIENTS

Fish tapeworm infestation is most common in Eastern Finland. Among 7 972 patients treated in the Central Hospital of Northern Karelia during the period from July 1 1958 to June 30 1959, 1 242 — 15 6 per cent — were tapeworm carriers Fish tapeworm infestation without the complication of anaemia was as a rule not taken as an indication of the need of admission to hospital Thus in most of these cases the tapeworm was an occasional finding In contrast patients suffering from tapeworm pernicious anaemia were admitted to hospital by reason of their anaemia. Accordingly no conclusion may be drawn from the present series on the incidence of tapeworm pernicious anaemia among tapeworm carriers in general

The incidence of tapeworm carriers given above is in agreement with that obtained in respect of the general population of Northern Karelia during the Red Cross campaign against the fish tapeworm (Vaittinen 1961) Nyberg (1960) found in Pielisjärvi — a rural district in Northern Karelia — a somewhat higher frequency (27 2 per cent) However none of these observations is as high as earlier calculations (50 to 100 per cent) on the occurrence of fish tapeworm in Eastern Finland (*Ehrström* 1926 Huhtala 1950)

The fish tapeworm carriers treated in the medical wards of the Central Hospital of Northern Karelia during the period February 1 to August 31 1959 were subjected to the *present study* with the exclusion of those patients with clear hypochromic sideropenic anaemia. In addition there were excluded all those too ill for treatment with tapeworm expulsion those who were non-cooperative, and those in whom the bone marrow aspiration failed The present series thus comprises 184 patients harbouring fish tapeworm The principal diseases of the tapeworm carriers without anaemia are listed in *Appendix II*

The following examinations were performed peripheral blood count sternal marrow puncture, assay of vitamin B_{12} in the serum, azur A resin test meal, neurological examination, and the (ordinary) Schilling test. The following drugs were utilized for worm expulsion *Extractum filicis* Alb Koponen (an extract of the rhizome of *Dryopteris filix mas Pharmacopoea Fennica* VIIth edition) Laparin, Medica (containing flavaspidic acid, and compounds of tin) Antiphen May and Baker (dichlorphen) or pumpkin seeds Cases 10 and 15 had an administration at home of vitamin B_{12} injections because of their delayed haematological response to

the worm cure, other cases were given no vitamin B_{12} treatment at home.

151 patients who had pathological or borderline Schilling test value, serum level of vitamin B_{12}, or number of large metamyelocytes in the sternal marrow by reason of the criteria used (and described in detail later) were asked to attend for re-examination two months after the expulsion of the worm. 95 persons accepted the invitation, and were subjected to the following examinations: examination of a stool specimen for tapeworm eggs, peripheral blood count, serum level of vitamin B_{12}, sternal marrow puncture, and neurological examination.

To facilitate analysis of the results, the material was divided into the following groups:

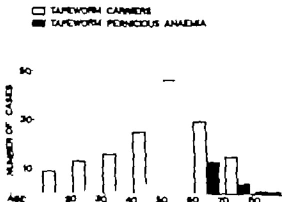
— 29 cases of tapeworm pernicious anaemia, with typical megaloblastic erythropoiesis in their sternal marrow;

— 155 cases of tapeworm carriers, with normoblastic erythropoiesis in their sternal marrow.

Age distribution.

The distribution of the present series according to age is presented in Figure. Twenty-four per cent of the tapeworm carriers and 7 per cent of the patients with tapeworm pernicious anaemia, were

Fig.
Diagram showing the age distributions of the tapeworm carriers and patients with tapeworm pernicious anaemia.



less than 40 years of age. Twenty-nine per cent of the tapeworm carriers, and 62 per cent of the patients with tapeworm pernicious anaemia, were more than 60 years of age. This difference is statistically significant ($P < 0.1$ per cent). The maximum peaks of the age distribution curves fell as regards tapeworm carriers between 50 and 60 years, and in cases of tapeworm pernicious anaemia between 60 and 70 years. Most of the cases of tapeworm pernicious anaemia in the present series were in patients of more advanced age. As for distribution by sex, no difference was found between tapeworm carriers without anaemia, and patients with tapeworm pernicious anaemia.

Seasonal distribution.

The tapeworm carriers in the present series were divided into three groups of approximately equal size according to

the month of examination. The seasonal distribution of tapeworm carriers and patients with tapeworm pernicious anaemia was as follows

Period	Tapeworm carriers	Patients with tapeworm pernicious anaemia
February to April	60 cases (39 per cent)	8 cases (28 per cent)
May and June	51 (33 ")	15 (52 ")
July and August	44 (28 ")	6 (20 ")
In total	155 cases	29 cases

An accumulation of cases of tapeworm pernicious anaemia in the period May and June is apparent in the present series in relation to the distribution of tapeworm carriers. However the monthly distribution of tapeworm pernicious anaemia undergoes considerable variations year by year In June 1960, only one case of tapeworm pernicious anaemia was seen in the same hospital wards.

METHODS USED

Peripheral blood count

The haemoglobin content of the blood was determined by employment of the oxyhaemoglobin method, the measuring apparatus being Beckman B spectrophotometer. The standard sample was analysed for iron content. The haematocrit values were found by centrifugation in capillary tubes. The red blood cell counts were made by means of Hellige haemoscope. The values below mil-lions per cub. mm., and the hite blood cells, were counted in the ordinary way in chamber. The differential counts of the white blood cells were made on stained coverslips (May-Grünwald-Giemsa) and the granulocyte counts calculated from the white blood cell, and differential counts.

Schilling test

During his studies on the absorption of itamin B_{12} with radioactive isotopes, Schilling (953) introduced urinary excretion test. In his original method, Schilling used test dose of µg of vitamin B_{12} labelled with ^{60}Co. Various doses have sub-sequently been used, and the effect of given amount of vitamin B_{12} on the amount absorbed has been demonstrated by Baker and Mollin (953). The proportion absorbed from the test dose decreases with an increase in the dose. With increasing dose, the absolute amount absorbed rises rapidly to certain level, and thereafter only slowly. The effect of the various test doses in the Schilling test appears to bear similarity to that in the faecal excretion test (Gräsbeck and Reisen-stein 957, Schilling 1958, Gräsbeck 960). Table presents variety of results obtained by various authors with different test doses administered to normal persons, and to patients suffering from Addisonian pernicious anaemia. Most investigators have utilized an amount of some 0.5 µg, which is commercially available in capsules. Following this test dose, most of the normal persons has shown urinary excretion of more than per cent, and patients with Addisonian pernicious anaemia less than 5 per cent of the radioactivity given.

In the present series, the Schilling test was carried out as follows: for period of three days the patient was given no drugs containing vitamin B_{12} or intrinsic factor. On the morning of the experi-ment he was given at 8 a.m., immediately after urination, an oral dose of 0.67 µg radioactive vitamin B_{12} with radioactivity of 0.5 µC of ^{60}Co (Racobalamin capsule, Abbot Laboratories, North Chicago, Ill.). At a.m., the patient was given mg of vitamin B_{12} parenterally as flushing dose. The urine was collected over 24 hours, and the amount of radioactivity excreted measured by means of scintillation counter (Frieseke Hoepfner with γ measuring end FH 41 /Za). The crystal was of NaJ(Tl) with diameter of 5 thickness of and zero effect of ca. 00 c.p.m. behind 50 mm lead. The standards containing 0, 5, 0, and 20 per cent of the whole test dose in tap ater were included in measurements each day. The measuring volume was litre. The impulses of the samples and standards were counted over minutes, and the background over an hour.

The Schilling test values in the present study were considered normal hen they were per cent or more. Those which were less than 5 per cent were considered to be pathological, and those between 5 and 9.9 per cent as borderline alues.

Serum level f vitamin B_{12}

So far assays of vitamin B_{12} in the serum have been carried out microbiologically in the majority

Table 1 Some results of Schilling tests, with various doses of radioactive vitamin B_{12}

Author	Dose μg	Healthy persons		Patients with pernicious anaemia	
		Number	Results	Number	Results
Hughes et al., 19 7	0 1	—	—	1	0.9
Allgén and Tornenius, 1958	0.5	55	12 —46	36	0 — 4
Mac Lean, 1955	0.57—0.9	10	6.7—31	6	< 1
Goldberg et al 1957	0 46	14	6.2—33.4	7	0 — 3
Miller et al 1957	0 48	16	17 —39	9	0 — 4
McIntyre et al 1956	0.5	12	> 15	20	0 — 4
Roth et al., 1957	0.5	33	8.4—28	29	0 — 6.9
Louis and Schwartz, 1957	0.5	33	10 —38	14	0 — 5
				1	5 —10
Schwartz et al., 1959	0.5	—		53	0 — 5
Dunn et al., 1958	0.5	—	—	15	0 — 3.9
Hutchinson et al 1958	0.5	15	17.5—46	13	0.34— 3.8
Oxenhorn et al., 1958	0.5	20	9 —36	15	0 — 1.2
Frick and Scheitlin, 1959	0.5	17	9 1—31	19	0 — 5.5
Mollin and Baker 1955	0 6	10	15.9—25.7	10	0 —10.5
Marlow et al., 1957	0.6	26	10.7—55.5	58	0 — 5
				2	5 6.9
Piney nd Stokes, 1958	0.6	22	9 —29	15	0 4 1
Pribilla et al 1958	0.6	20	10.3—35	10	0 — 3
Gehrman, 1960	0 6	16	10.5—26.5	20	0 2.9
Smythe 1957	0 75	10	11 —26	3	0.25—
Boll and Mehl, 1959	0.83	4	13.9—16	28	0 — 4.6
Ebbesen 1958	1 0	110	12 — 42	38	0 — 5
				4	5 — 6 6
Nyberg et al 1958	0 5 2.0	50	4.2 – 30	—	
Gräsbeck et l 1956	1 — 2	11	> 12.6	18	0 1
Schilling 1953	.0	—	—	6	0 — 3.8
Best et al., 1954	2.0	4	9.2—14 4	10	0.2
Schilling et al 1955	2 0	18	7 22	35	0 — .3
Ellenbogen et al 1955	2 0	17	5.4—17	24	0 2 4
Rabiner et al., 1956	2.0	24	3.0 – 34.2	56	0.3 – 3.0
Toporek and Brandborg 1955	2.34			21	0.2 – 1.8
Klayman et al. 1955	3 0	3	7.9 – 12	7	0 1
Berlyne et al 1957	3.0	5	4.0— 7 1	10	0.3 1

of cases, the test organism being *Euglena gracilis* (Hutner et al. 1948, Ross 950) nd *Lactobacillus leichmannii* (Hoffman et al. 1948) There has been some inconsistency in the methods employed, and the results obtained by the various investigators ha e been divergent. Girwood (954) obtained in doubl determinations with both of these micro-organisms values which were comparable T ble a present list of serum levels of itamin B_{12} obtained by arious investigators in healthy persons, and in patients with Addisonian pernicious anaemia, w th *Euglena gracilis* being utilized as the test organism. Despite the arious modifica ions of method, the serum levels of itamin B_{12} are as regards most patients with Addisonian per nicious anaemia within a range of 0 to 100 μμg per ml. On occasion, values between 00 and 200 μμg per ml serum have bo been found

Table 2. Some results of the assay of vitamin B_{12} in the serum, using E g l z III as test organism.

Author	Healthy persons		Patients with pernicious anaemia	
	Number	Range µµg per ml	Number	Range µµg per ml
Ross, 1950	12	350—750	4	< 10
Mollin and Ross, 1952	123	60—720	43	< 16—103
Mollin and Ross, 1954	126	100—900	190	< 100
Mollin and Ross, 1957	223	100—900	320	< 10—100
Pitney and Beard, 1953	—		10	< 20
Beard et al., 1954	22	86—460	—	
Pitney and Beard, 1954	57	86—460	—	
Pitney and Beard, 1955	—		17	0—46
Nicholas and Pitney 1958	218	190—875	—	
Killander 1953	56	120—710	12	< 25—103
Killander 1957	156	130—1200	167	< 100
			11	100—199
			2	200—399
			2	> 400
Lear et al., 1954	20	293—836	53	0—83
Uchino, 1957	23	67—200	—	
Nyberg, 1960	48	< 100		
	907	> 100		

these cases. On the other hand, some normal subjects have shown values below 00 µµg per ml.

In the present series, the assay of vitamin B_{12} in serum was carried out according to the following modification of Hoff-Jørgensen (954) method:

The test organism was *Euglena gracilis*, strain Z. The apparatus used in the culture of the test organism was regular thermostat case, and the necessary light was obtained from two fluorescent lamps of 40 watts each, built into the upper part of the case. The test tubes were held in racks, and covered with foil of aluminium. After each time of use, all the glassware was treated with dichromate-sulphuric acid solution, in order to eliminate possible traces of organic material. The turbidity as measured by means of Beckman B spectrophotometer. The basic medium used had the following composition (double strength):

Potassium dihydrogen phosphate (KH_2PO_4)	g
DL-malic acid	g
L-glutamic acid	g
Magnesium sulphate ($MgSO_4$, 7 H_2O)	.0 g
Zinc sulphate ($ZnSO_4$, 7H_2O)	88 mg
Calcium carbonate ($CaCO_3$)	100 mg
Ferrous sulphate ($FeSO_4$ 7 H_2O)	30 mg
Manganese sulphate ($MnSO_4$, 4H_2O)	6.2 mg
Cupric sulphate ($CuSO_4$, 5 H_2O)	3.9 mg
Cobalt sulphate ($CoSO_4$, 7H_2O)	7 mg
Sodium molybdate (Na_2MoO_4, 2H_2O)	.0 mg
Boric acid (H_3BO_3)	22.2 mg
Thiamine hydrochloride	2.0 mg
Water to	000 ml

After the components had been dissolved, the pH was adjusted to 3.8 and the solution was filtered, bottled, and autoclaved. Before use, per cent of glucose as sterile solution was added.

The stock culture of *Euglena gracilis* was grown once week in tubes containing 5 ml of the double strength medium, and 5 ml of solution of 00 µµg vitamin B_{12} per ml.

The standards were made from U.S.P. Reference Standard preparation of vitamin B_{12}. The stock solution, containing 0. µg per ml, was divided into ampoules of ml each. These were closed, autoclaved, and stored at temperature of —20 C. New standards were made from the stock solution for each batch. Their final concentrations were 50, 25, 3.6 2 5 .8, 8, and µµg per ml. Four tubes of each concentration were set in every batch.

The samples of serum were stored at —20 C prior to analysis. For the assay distilled water was added to obtain a dilution of the serum of 1 to 10. 0.5 ml of the medium was added to 0.5 ml of the dilution of the sample, or the standard. The tubes were then kept in a boiling water bath for 15 minutes, and inoculated after cooling The inocula was 0.05 ml of a dilution made in a proportion 1 to 10 from the subculture of *Euglena* grown in 5 days. A double determination was carried out for each sample. The tubes were then incubated for 8 days at + 30 in light.

The growth response was measured after filling the tubes to 10 ml, and following vigorous shaking of the suspension to achieve homogeneity The optical density was measured in 10 mm couvettes at a wave length of 600 μ

The lower limits of sensitivity of the method in the determination of the vitamin B_{12} in serum are reached at 10 μμg per ml by means of *Euglena*. On the other hand, when the serum is diluted to a proportion of only 1 to 20, as here, the high values near 1,000 μμg per ml cannot be distinguished from each other Nevertheless, they are clearly bove the lower pathological range

The accuracy of the method was checked by making double determinations of 39 samples in different batches. The standard deviation of the differences of the double values from the mean values was 39 per cent. To eliminate at least to some extent the effect of a possible drift in the values of the various batches, the samples were placed in a big jar and a random selection taken for determination. Accordingly technical variations should not be the cause of any possible seasonal divergences. Killander (1957) using another modification of vitamin B_{12} assays with *Euglena gracilis* as the test organism, had a corresponding standard deviation of the double values of 21 5 per cent. However the batches showing drift of the values had been excluded.

In what follows below values of vitamin B_{12} higher than 200 μμg per ml have been regarded as normal. Values less than 100 μμg per ml have been considered as pathological, and thus the remaining results (100 to 200 μμg per ml) represent borderline values.

The bone marrow

The present series of patients wa divided into groups according to the occurrence of typical megaloblastic erythropoiesis in the bone marrow the megaloblasts were recognized by the same criteria as those employed by Herbert (1959) The present series was composed in such a manner that all cases of tapeworm pernicious anaemia had typical megaloblastic erythropoesis. In the present study attention was devoted particularly to changes in the myelopoiesis of the tapeworm carriers and patients suffering from tapeworm pernicious anaemia. The first signs of a possible change from normoblastic towards megaloblastic erythropoiesis in the tapeworm carriers were also observed.

Megaloblastic erythropoiesis in Addisonian pernicious anaemia was established by Zadek (1921) and in tapeworm pernicious anaemia by Tötterman (1933) Changes in the erythropoiesis, interpreted as incipient megaloblastic maturation disturbance have been reported, and the terms used include atypical megaloblastic maturation (Downey 1932) intermediate megaloblasts (Mollin and Dacie 1950, Bastrup-Madsen 1952, 1954, 1956, Jewsbury 1954, Mollin and Ross 1954, Hansen 1960) partial megaloblastic erythropoesis (Pedersen et al. 19,7) megaloblastoid cell forms, and macronormoblasts (Herbert 1959)

The samples of bone marrow utilized in the present work were obtained by means of a sternal puncture and stained on ordinary coverslips with May-Grunwald-Giemsa stain. A diameter of 100 metamyelocytes was measured in each case. The number of cells exceeding 14 μ was noted, and the mean diameter of the 100 cells measured was calculated.

In this investigation, metamyelocytes exceeding 14 μ were regarded as normal if amounting to less than 30 per cent of the whole, cases of 50 per cent or above regarded as pathological, and those in the range 30 to 49 taken as borderline alues.

Neurological examination

The neurological examination was in the main carried out according to the scheme followed in the Neurological Department of the University Central Hospital, Helsinki. The vibration sense was studied quantitatively by the utilization of a tuning fork C° of 128 vibrations per second, and weight of 72.8 g. The examination was carried out according to the description by Björkenheim (1951) The normal series consisted of 60 hospital patients without tapeworm or neurological disorders, nd

their age distribution was similar to the tapeworm carriers. The lower limit of the normal alues was obtained by drawing median line for the results in each age group. Ten seconds was then subtracted from the values. The sense of position in the hands and feet was tested by asking the patient, ho had his eyes closed, to name the finger or toe touched by the observer. If the criteria used by Björkenheim (95) were accepted as such in the present series there would have been disturbances in the sense of position of the fingers in 36, and in that of the toes in 49 per cent of the tapeworm carriers. In the present series, three errors as regards the fingers, *and four in the toes, were taken* as allowable in all age groups.

The neurological symptoms were not studied in patients suffering from ailments known to cause neurological disorders. Those unable to provide sufficient cooperation were also excluded.

Azur A resin test meal

The azur A resin test as effected as follows: after an overnight fast, the patient was given 200 mg of caffeine. The urine excreted during the following four hours was taken as blind sample. Then an amount of grams of azur A resin crystals as given, and the quantity of azur A excreted in the urine during the two succeeding hours measured photometrically. Values of 0.6 mg or more were accepted as normal, and those less than 0.3 mg considered as representing achlorhydria (A.M.A. Council 959)

Statistical methods

In making comparisons of the relati fre quencies of occurrence in the arious groups, the ariable

$$\frac{p_1 - p_2}{\sqrt{p(\ -p)\left(\frac{1}{\ } + \frac{1}{n_2}\right)}}$$

was used, and the assumption made that it obeyed the normal distribution. Here p_1 and p_2 represent the frequencies for comparison, p the mean fre quency n_1 the number of all observations in the group, of which p_1 is part, and n_2 the corresponding number attached to p_2

The *standard deviation of the double assay* of itamin B_{12} was determined as follows. The stochastic variables used were the values obtained divided by the corresponding mean alues of each pair It was assumed that such reduced ariables had the same deviation. Each pair of variables and *y* had the same mean = The standard deviation was then calculated.

$$SD = \sqrt{\frac{\ }{n-}\ (\ -\ )}$$

THE RESULTS OBTAINED, GIVEN SEPARATELY FOR *EACH METHOD USED*

The peripheral blood count

The distributions of haemoglobin values red blood cell counts and granulocyte counts in tapeworm carriers and in patients with tapeworm pernicious anaemia, are presented in Figures 2 3 and 4 The criterion of tapeworm pernicious anaemia in the present series of patients was the typical megaloblastic erythropoiesis in the bone marrow not the possible anaemia As can be seen in Figures 2 and 3 all the patients in the tapeworm pernicious anaemia group had haemoglobin values which were less than 10 g per 100 ml and red blood cell counts of less than 3 500 000 per cub mm In addition to the anaemia, they showed marked granulocytopenia Only one tapeworm carrier had a haemoglobin value of less than 10 g per 100 ml, and two worm carriers had red blood cell counts of less than 3 000 000 per cub mm. No tendency was found towards higher haemoglobin values or red blood cell counts at the follow up examination of the tapeworm carriers after worm expulsion when this group was taken as a whole. Every tapeworm pernicious anaemia patient examined two months after worm expulsion (and Schilling test) showed a rise in haemoglobin value and red blood cell count, irrespective of the presence of tapeworm eggs in the stool The tapeworm pernicious anaemia patients showed a marked granulocytopenia As regards tapeworm carriers, the granulocytopenia was not so marked but subsequent to a successful expulsion of the worm, they displayed a slight tendency towards higher granulocyte counts, as can be seen in Figure 4. The numbers of the eosinophilic cells were calculated from the differential counts of the white blood cells. The counts in the patients with tapeworm pernicious anaemia, and in the tapeworm carriers before and after worm expulsion showed no significant differences.

Azur A resin test meal

Table 3 contains a summary of the results of the azur A resin test meals. There were more achlorhydrics (test value less than 0 3 mg) among the tapeworm pernicious anaemia patients (52 per cent) than among the tapeworm carriers (16 per cent) The difference between the frequencies is statistically significant (P < 0 1 per cent) As regards the cases with values which were suggestive of achlorhydria, in 10 cases the azur A resin test was repeated two months after successful worm expulsion. There was no re-appearance of free hydrochloric acid in any of them.

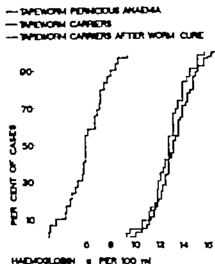


Fig. 2.

Diagram showing the cumulative distributions of the haemoglobin values in g per 100 ml of patients with tapeworm pernicious anaemia and tapeworm carriers.

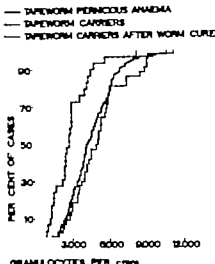


Fig. 4.

Diagram showing the cumulative distributions of the granulocyte counts per c.mm. in patients with tapeworm pernicious anaemia and tapeworm carriers.

Fig. 3.

Diagram showing the cumulative distributions of the red blood cell counts in 10^6 per mm. in patients with tapeworm pernicious anaemia and tapeworm carriers.

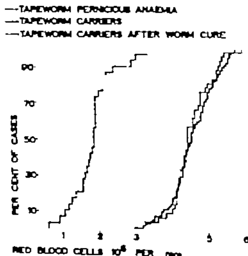


Table 3 Results f a..ur 1 res test meal given to 29 patients with tapeworm pernicious anaemia and 154 tapeworm carriers

Anae resin test value	Patients with tapeworm pernicious anaemia		Tapeworm carriers	
	Number	Per cent	Number	Per cent
≥ 0 6 mg	3	17	102	68
0.3—0.59 mg	9	31	25	16
< 0.3 mg	15	52	5	16
Total	29	100	154	100

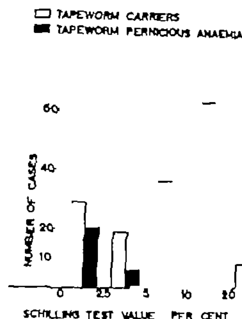


Fig 5.
Diagram showing the distributions of the Schilling test values in patients with tapeworm pernicious anaemia nd t peworm carriers.

Schilling test

The distribution of the Schilling test values among the patients in this series is presented in Figure 5 Among the 29 cases of tapeworm pernicious anaemia the values were

o to 4 9 i 26 cases (90 per cent) and
5·0 t 9 9 in 3 cases (10 per cent)

Not one of them showed normal values in the Schilling test Among the 155 tapeworm carriers, the Schilling test values were

o to 4·9 in 48 cases (31 per cent),
5·0 t 9 9 in 36 cases (23 per cent) and
10.0 or more in 7 cases (46 per cent)

Pathological Schilling test values in various age groups of tapeworm carriers were as follows

up to 39 year 8 cases out of 38 (21 per cent)
40 to 59 years 21 cases out of 72 (29 per cent) nd
60 years and elder 19 cases out of 43 (4 per cent)

There was found a tendency towards lower Schilling test values with advancing age among the tapeworm carriers The percentages of the pathological Schilling test values in the youngest and the oldest group differ from each other statistically to an almost significant extent ($P <$ 5 per cent)

No difference was observable between the sexes in the distribution of the Schilling test values and no seasonal variation of the Schilling test values occurred among the patients.

Serum level of vitamin B_{12}

The values obtained in the assays of vitamin B_{12} in serum are presented in Figure 6. The following results ere obtained in respect of the 29 patient with tapeworm pernicious anaemia

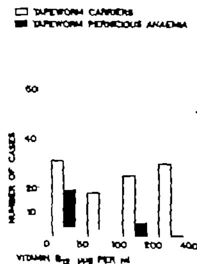


Fig. 6.

Diagram showing the distributions of the serum levels of vitamin B_{12} in tapeworm carriers and patients with tapeworm pernicious anaemia

to 100 in 32 cases (76 per cent)
to 200 in 6 cases (per cent), and
more than 200 in case (3 per cent)

Among the 55 tapeworm carriers, the corresponding findings were as follows

to 100 in 49 cases (32 per cent)
to 200 in 25 cases (6 per cent) and
more than 200 in 8 cases (52 per cent).

As the standard deviation from the mean values of the double determinations was 39 per cent, there is some doubt whether the results obtained from the tapeworm carriers were due only to methodological errors. On the assumption that the serum level of vitamin B_{12} had in fact been the same (200 μμg per ml) in all the 155 tapeworm carriers, the results were calculated by employment of the standard deviation of the method. The number of such arbitrary pathological values (10 per cent) is statistically less to a significant extent ($P < 0.1$ per cent) than the number (32 per cent) obtained in the assays. Accordingly the low values obtained from the present series of patients cannot be ascribed to methodological errors.

Two months after successful worm expulsion (and Schilling test) an assay of vitamin B_{12} in serum was carried out for 44 tapeworm carriers. The results of



Fig. 7

Diagram showing the distributions of the serum levels of vitamin B_{12} in 44 tapeworm carriers before expulsion of the worm and two months later

these assays are presented in Figure 7. The values were

	before worm cure	after worm cure
to 100 in	8 cases (41 per cent)	cases (5 per cent)
10 to 200 in	6 cases (4 per cent)	5 cases (per cent)
more than 200 in	20 cases (43 per cent)	37 cases (84 per cent)

Table 3 Results of azur A resin test meals given to 29 patients with tapeworm pernicious anaemia and 154 tapeworm carriers

Azur resin test value	Patients with tapeworm pernicious anaemia		Tapeworm carriers	
	Number	Per cent	Number	Per cent
≥ 0 6 mg	5	17	102	68
0.3—0.59 mg	9	31	25	16
< 0.3 mg	15	5..	5	16
Total	29	100	154	100

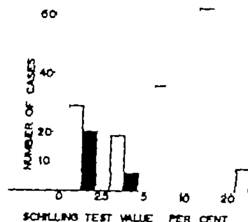


Fig. 5.
Diagram showing the distributions of the Schilling test values in patients with tapeworm pernicious anaemia and tapeworm carriers.

Schilling test

The distribution of the Schilling test values among the patients in this series is presented in Figure 5. Among the 29 cases of tapeworm pernicious anaemia, the values were

o to 4.9 in 26 cases (90 per cent) and
5.0 to 9.9 in 3 cases (o per cent)

Not one of them showed normal values in the Schilling test Among the 155 tape worm carriers the Schilling test values were

to 4.9 48 cases (3 per cent
5 0 to 9 9 in 36 cases (23 per cent) and
0.0, or more in 71 cases (46 per cent)

Pathological Schilling test values in various age groups of tapeworm carriers were as follows

up to 39 years 8 cases out of 38 (21 per cent)
40 to 59 years 21 cases out of 72 (29 per cent) nd
60 years and older 9 cases out of 45 (42 per cent)

There was found a tendency towards lower Schilling test values with advancing age among the tapeworm carriers The percentages of the pathological Schilling test values in the youngest and the oldest group differ from each other statistically to an almost significant extent (P < 5 per cent)

No difference was observable between the sexes in the distribution of the Schilling test values, and no seasonal variation of the Schilling test values occurred among the patients.

Serum level of vitamin B_{12}

The values obtained in the assays of vitamin B_{12} in serum are presented in Figure 6 The following results were obtained in respect of the 29 patients with tapeworm pernicious anaemia

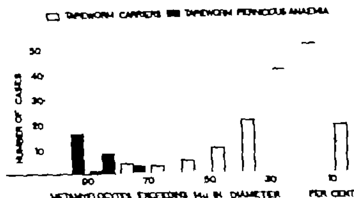


Fig. 8.
Diagram showing the distribution of percentages of metamyelocytes exceeding 14 μ in diameter in tapeworm carriers and patients with tapeworm pernicious anaemia.

in Figure 8. All of the 29 tapeworm pernicious anaemia patients in the series had more than 30 per cent of large metamyelocytes in their marrow specimens. Among the 155 tapeworm carriers, 13 cases (8 per cent) showed such pathological values. 31 tapeworm carriers (20 per cent) had borderline numbers of large metamyelocytes, and 111 cases (72 per cent) were normal.

Two months after a successful worm expulsion and Schilling test, a new sternal marrow specimen was obtained from 42 tapeworm carriers, and from 19 tapeworm carriers in whom the worm cure had failed. In no case was there found more than 29 per cent of large metamyelocytes.

The mean diameter of the 100 metamyelocytes measured on each specimen was also calculated. The mean diameter of the metamyelocytes exceeded 14 μ in all of the 29 tapeworm pernicious anaemia patients of the series. In 23 cases (15 per cent) of the 155 tapeworm carriers, the mean diameter of the metamyelocytes exceeded 14 μ. At the follow-up examination, not one of the 42 former tapeworm carriers showed a mean metamyelocyte diameter of more than 14 μ.

In the various age groups, some differences were noted with regard to the number of the large metamyelocytes. In two cases out of 38 (5 per cent) pathological values were obtained in the group of less than 40 years of age. In the group of patients of 60 years and above, the corresponding figure was 5 cases out of 45 (11 per cent). Nevertheless, the difference is not statistically significant.

Neurological disturbance

In the neurological examination, the most positive findings were obtained in the following tests

Paraesthesias.

	Tapeworm anaemia (23 cases)	Tapeworm carriers (38 cases)
in upper limbs	6 cases	4 cases
in lower limbs	8 cases	43 cases
in total	8 cases (35 per cent)	43 cases (3 per cent).

The difference between the percentages of the pathological serum levels of vitamin B_{12} before and after successful worm expulsion is statistically significant ($P < 0.1$ per cent). On the contrary, the vitamin B_{12} assay performed subsequent to unsuccessful worm cure in 24 tapeworm carriers showed no significant diminution in the number of the pathological values. Thus the flushing dose of vitamin B_{12} given in the Schilling test was incapable of maintaining normal serum levels of vitamin B_{12} two months after an unsuccessful worm cure.

In the various age groups of the tapeworm carriers, there were found pathological values of vitamin B_{12} in the serum as follows:

up to 39 years 9 cases out of 38 (24 per cent)
40 to 59 years 22 cases out of 72 (31 per cent) and
60 years and elder 8 cases out of 45 (40 per cent)

A tendency towards lower levels of vitamin B_{12} in the serum with advancing age appeared to exist among the tapeworm carriers. However, the difference is not statistically significant. The sex of the patient did not give rise to differences in the vitamin B_{12} assays.

The seasonal variation of the pathological vitamin B_{12} values in the tapeworm carriers was:

February to April 29 cases out of 60 (48 per cent)
May and June 11 cases out of 51 (21 per cent) and
July and August 9 cases out of 44 (20 per cent)

The difference in the percentages of pathological serum B_{12} vitamin levels obtained during the first and the last seasonal period of the study is statistically significant ($P < 1$ per cent). By inclusion of the tapeworm pernicious anaemia patients, the following figures were obtained in respect of the pathological values: February to April, 51 per cent out of 68 cases, May and June 36 per cent out of 66 cases and July and August 24 per cent out of 50 cases. Also in this comparison, the difference between the first and the last period is statistically significant ($P < 1$ per cent).

Erythropoiesis

The erythropoiesis was typically megaloblastic in all of the 29 cases of tapeworm pernicious anaemia of the present series, by reason of the criteria adopted for the diagnosis. Among the 155 tapeworm carriers, features interpreted as incipient megaloblastic change of the erythropoiesis were recorded in 14 cases (9 per cent). In these cases the erythropoiesis was mainly normoblastic. At the mature end of the erythroblastic series, some cells displayed disturbed maturation of the nuclei in comparison with the cellular plasm. In addition, the size of the cytoplasm was large. No signs of megaloblastic erythropoiesis were observed at the re-examination, irrespective of whether the worm expulsion had proved successful or not.

Large metamyelocytes

The percentages of the metamyelocytes which exceed 14 μ in diameter are shown

DISCUSSION OF THE RESULTS

The acid A resin method gave a relatively high incidence of gastric achlorhydria among the patients with tapeworm pernicious anaemia constituting the present series. The figure thus obtained may be too high, as the caffeine stimulation used is not maximal for the gastric secretion (*A.M.A. Council* 1959). Nevertheless, the results are in accord with the earlier observations reviewed above (Schauman 1894, Grönberg 1912, Schauman and Levander 1917, Helander 1945, Lumme et al. 1954, Siurala 1954, and Gräsbeck 1955).

The low Schilling test values found in the patients of the present series with tapeworm pernicious anaemia agree with those determined earlier (Karpainen and Ohela 1957, Brante and Ernberg 1958, Nyberg et al. 1958, Nyberg 1959, 1960). However, among the tapeworm carriers there seem to be Schilling test values (46 per cent) which more obviously approach the normal than those obtained in the earlier series. All of the 8 tapeworm carriers reported by Gräsbeck et al. (1956) had Schilling test values of less than 10 per cent. If a calculation is made from the diagram presented by Nyberg et al. (1958), only 8 per cent of their 50 tapeworm carriers had normal Schilling test values (more than 10 per cent) and 40 per cent were on the borderline (between 5 and 10 per cent). The difference is probably explained by the larger test doses (0.5 to 2 μg) of radioactive vitamin B_{12} used by Nyberg et al. In their discussion, Nyberg et al. (1958) were of opinion that their results were in complete agreement with those obtained in earlier investigations (Nyberg 1958) which indicated that the Finnish *D. latus* always impairs the vitamin B_{12} absorption of its hosts. In the present series, one third of the tapeworm carriers showed clearly impaired absorption of vitamin B_{12}, as judged by the Schilling test.

The low serum levels of vitamin B_{12} in the patients with tapeworm pernicious anaemia in the present series are in accordance with earlier observations. The 20 cases previously reported (Nyberg and Östling 1956, Killander 1957, Brante and Ernberg 1958, Nyberg 1960) all had serum levels of vitamin B_{12} of less than 100 μμg per ml. In the series of 361 tapeworm carriers, Nyberg (1960) found a pathological condition in 51.5 per cent. When the 8 cases of tapeworm pernicious anaemia in his series are excluded, 50.5 per cent remain; this seems a rather high percentage in comparison with the present series. As Nyberg investigated his series in the spring (1958) it is easy to see that the

Objective signs of incoordination.

	Tapeworm anaemia	T peworm carriers
n upper limbs	3 cases	15 cases
in lower limbs	none	4 cases
in total	3 cases (5 per cent)	15 cases (1 per cent)

Pyramidal signs

	T pew rm anaemia	Tapeworm carriers
Increased reflexes		
knee jerk	4 cases	14 cases
ankle jerks	4 cases	11 cases
Extensor plantar response	3 cases	7 cases
Clonus	2 cases	1 case
In total	4 cases (7 per cent)	18 cases(13 per cent)

Impaired postural sense·

	T peworm anaemia	T peworm carriers
in upper limbs	3 cases (13 per cent)	6 cases (4 per cent)
in lower limbs	5 cases (22 per cent)	7 cases (5 per cent)

Impaired ibration sense

	T peworm naemia (20 cases)	T peworm carriers (34 cases)
in upper limbs	5 cases (25 per cent)	26 cases (9 per cent)
in lower limbs	4 cases (20 per cent)	23 cases (7 per cent)

In all deep sensibility was impaired in 57 per cent of the tapeworm pernicious anaemia patients, and 34 per cent of the tapeworm carriers

Among the present series, no abnormal neurological symptom or sign was detectable in three patients with tapeworm pernicious anaemia (13 per cent) and in 53 tapeworm carriers (38 per cent)

At the follow up examination, improvement in the neurological disturbance of the tapeworm pernicious anaemia patients and tapeworm carriers was seen in 13 cases out of 44

DISCUSSION OF THE RESULTS

The arur A resin method gave relatively high incidence of gastric achlorhydria among the patients with tapeworm pernicious anaemia constituting the present series. The figure thus obtained may be too high, as the caffeine stimulation used is not maximal for the gastric secretion (A.M.A. Council 1959) Nevertheless, the results are in accord with the earlier observations reviewed above (Schauman 1894, Grönberg 1912, Schauman and Levander 1917 Helander 1945, Lumme et al 1954, Siurala 1954 and Gräsbeck 955)

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Objective signs of incoordination

	T peworm anaemia	T peworm carrier
In upper limbs	3 cases	15 cases
In lower limbs	none	4 cases
In total	3 cases (3 per cent)	15 cases (11 per cent)

Pyramidal signs:

	T peworm naemia	Tapeworm carriers
Increased reflexes		
knee jerks	4 cases	14 cases
ankle jerk	4 cases	1 cases
Extensor plantar response	3 cases	7 cases
Clonus	2 cases	1 case
In total	4 cases (17 per cent)	18 cases(13 per cent)

Impaired postural sense

	Tapeworm anaemia	Tapeworm carriers
n upper limbs	3 cases (13 per cent)	6 cases (4 per cent)
in lower limbs	5 cases (22 per cent)	7 cases (5 per cent)

Impaired bration sense

	Tapeworm anaemia (20 cases)	Tapeworm carriers (34 cases)
In upper limbs	5 cases (25 per cent)	26 cases (19 per cent)
in lower limbs	4 cases (20 per cent)	03 cases (7 per cent)

In all deep sensibility was impaired in 57 per cent of the tapeworm pernicious anaemia patients and 34 per cent of the tapeworm carriers.

Among the present series, no abnormal neurological symptom or sign was detectable in three patients with tapeworm pernicious anaemia (13 per cent) and in 53 tapeworm carriers (38 per cent)

At the follow up examination, improvement in the neurological disturbance of the tapeworm pernicious anaemia patients and tapeworm carriers was seen in 13 cases out of 44

SUMMARY OF THE RESULTS

In order to obtain survey of the distributions of the values determined by the various methods used, the most important of them have been combined in Table 4. On mutual comparison of the results, the following comments may be made:

— the distributions of the values obtained by means of the Schilling test and the serum assay of vitamin B_{12} were of the same magnitude. Most of the cases with tapeworm pernicious anaemia presented pathological values in both respects, and some were on the borderline. Some 30 per cent of the tapeworm carriers had pathological, 20 per cent borderline, and 50 per cent normal values. The pathological values in the Schilling test and the serum assay of vitamin B_{12} were the commonest signs of disturbed vitamin B_{12} metabolism among the tapeworm carriers.

Table 4. Summary of the results obtained by different methods: Schilling test, serum assay of vitamin B_{12}, measurement of the metamyelocytes, haemoglobin determination, examination of the deep sensibility and azur A resin test used

Method	133 patients with tapeworm pernicious anaemia			39 tapeworm carriers		
	Pathological per cent	Borderline per cent	Normal per cent	Pathological per cent	Borderline per cent	Normal per cent
Schilling test	90	10	—	31	23	46
Serum B_{12}	76	21	3	32	16	52
Metamyelocytes	100	—	—	8	20	72
Haemoglobin	100	—	—	1	19	80
Deep sensibility	57		43	35		65
Azur A	52	31	17	16	16	68

The limits of the various groups were:

	Pathological	Borderline	Normal
Schilling test	less than 5.0	5.0 to 9.9	10.0 per cent and above
Serum B_{12}	less than 101	101 to 200	more than 200 $\mu\mu g$ per ml
Metamyelocytes	30 and more	30 to 49	less than 30 per cent exceeding 14 μ
Haemoglobin	less than 10.0	10.0 to 11.9	12.0 g and above per 100 ml
Deep sensibility	see the text		
Azur A	less than 0.3	0.3 to 0.59	0.6 mg and above

results in the present instance were the same during the corresponding season. During the summer months, pathological vitamin B_{12} levels in serum existed in only 20 per cent of the present series of tapeworm carriers. It appears probable that the serum level of vitamin B_{12} in tapeworm carriers undergoes seasonal variations. During the months of winter and spring the values below normal were more than during the summer. Methodological errors compel caution in drawing conclusions.

Metamyelocytes exceeding 14 μ in diameter were present to the extent of at least 70 per cent in all the 5 patients with tapeworm pernicious anaemia reported on by Heinivaara and Kalpainen (1960). Of their 6 tapeworm carriers, in three cases the percentages were between 30 and 50, and in three cases less than 30. In their discussion, Heinivaara and Kalpainen expressed the view that the gap they had observed in the 50—70 per cent range could be filled in a major series; the present study has accomplished this. As the percentages of large metamyelocytes fell within this range (50 to 70 per cent) in two cases of tapeworm pernicious anaemia with fully developed megaloblastic erythropoiesis, these values were regarded as pathological in the present series.

Neurological disturbance in patients suffering from tapeworm pernicious anaemia, and in tapeworm carriers, has been most extensively studied by Björkenheim (1951). His material included 95 patients with tapeworm pernicious anaemia and 30 tapeworm carriers. There was thus a marked difference from the present series. However, some comparisons may be made. The figures concerning incoordination and pyramidal signs correspond closely as regards patients with tapeworm pernicious anaemia. The other figures do not differ from each other to the extent that they cannot be explained by the difference in size of the series and by the examination being effected by another investigator. As regards the tapeworm carriers, the number of neurological disturbances in the present series (62 per cent) seems higher than in that studied by Björkenheim (33 per cent). Further to the sources of differences mentioned above, it must be pointed out that the major group of neurological disturbances found in the present series were constituted by paraesthesias. These are extremely unreliable as subjective symptoms as a basis for drawing conclusions.

INTERRELATIONS OF THE SIGNS OF VITAMIN B_{12} DEFICIENCY OBTAINED BY VARIOUS METHODS

The serum level of vitamin B_{12} and the Schilling test are the most commonly used criteria of a disturbed vitamin B_{12} metabolism. However it should be borne in mind that the Schilling test does not measure the vitamin B_{12} deficiency but only the absorption of the vitamin. The vitamin B_{12} stores may be adequate to maintain a normal serum level of vitamin B_{12}, despite Schilling test values which are obviously low over a number of years. On the other hand, the serum assay of vitamin B_{12}, even if it reflects the vitamin B_{12} concentration in the organism, has been criticized from a methodological viewpoint.

Megaloblastic erythropoesis when it occurs in cases with vitamin B_{12} deficiency — and excluding other causes — is generally accepted as a proof of a fully developed deficiency state. The megaloblasts are easily and clearly identifiable morphologically. In the present series of patients, possible causes of megaloblastic erythropoesis other than vitamin B_{12} deficiency were ignored. For these reasons, the serum levels of vitamin B_{12} and the Schilling test values have been compared with the megaloblast phenomenon, the most reliable clinical indication of vitamin B_{12} deficiency.

The Schilling test, the serum assay of vitamin B_{12}, and the presence of megaloblasts

Comparison of the serum levels of vitamin B_{12} with the occurrence of megaloblasts in respect of the whole present series of 184 tapeworm carriers and patients with tapeworm pernicious anaemia, gave the following results.

The serum level of vitamin B_{12} was pathological in 71 patients, in whose bone marrow were found

fully developed megaloblasts in 23 cases (3 per cent)
megaloblastic features in 9 cases (3 per cent), and
no signs of megaloblasts in 39 cases (56 per cent)

In the 31 cases with borderline values of vitamin B_{12} in the serum, bone marrow examination established

fully developed megaloblasts in 6 cases (9 per cent)
megaloblastic features in case (3 per cent), and
no signs of megaloblasts in 24 cases (78 per cent)

— the deep sensibility was impaired in more than one half of the patients with tapeworm pernicious anaemia. About one third of the tapeworm carriers showed similar signs of deficiency in vitamin B_{12}.

— the number of metamyelocytes exceeding 14 μ in diameter was clearly pathological in all the cases of tapeworm pernicious anaemia. Only 8 per cent of the tapeworm carriers without megaloblasts had a pathological number of large metamyelocytes.

— features of megaloblastic erythropoiesis were found in 9 per cent of tapeworm carriers.

— the haemoglobin content of the peripheral blood was less than 10 g per 100 ml in all the cases of tapeworm pernicious anaemia in the series.

— the azur A resin test meal gave values suggesting gastric achlorhydria in 52 per cent of the patients suffering from tapeworm pernicious anaemia. The finding was similar among tapeworm carriers in only 16 per cent of cases.

The Schilling test and the serum level of vitamin B_{12}

When the 184 tapeworm carriers and the patients with tapeworm pernicious anaemia of this series were taken combined in one group, the comparative results of the Schilling tests and assays of vitamin B_{12} were as follows

The Schilling test value was pathological in 74 cases, in which the serum level of vitamin B_{12} was

pathological in 4 cases (35 per cent)
on the borderline in cases (5 per cent) and
normal in [illegible]2 cases (50 per cent)

The Schilling test showed borderline values in 59 cases. Their serum levels of vitamin B_{12} were

pathological in cases (3 per cent)
on the borderline in 0 cases (26 per cent) and
normal in 7 cases (43 per cent)

The Schilling test value was normal in 71 cases. In this group the serum level of vitamin B_{12} was

pathological in 8 cases (15 per cent)
on the borderline in cases (4 per cent) and
normal in 43 cases (6 per cent)

The differences in the occurrence of the pathological serum levels of vitamin B_{12} in the cases with pathological and those with normal Schilling test values show significant differences ($P < 0.1$ per cent) in the present series as a whole.

As there were 25 per cent pathological serum levels of vitamin B_{12} among the tapeworm carriers without impaired vitamin B_{12} absorption which could be detected by means of the Schilling test, doubt might exist whether this was occasioned only by methodological errors in the assay of itamin B_{12}. In 25 cases the difference of the two double deter minations in various batches was less than 33 per cent from the mean, which might here be accepted as quite reliable. Of these, the Schilling test value was normal in 11 cases, which had normal serum levels of vitamin B_{12} in 5 cases, on the borderline in 3 cases, and pathological in 3 cases. As can be seen, the 25 per cent of the cases with a discrepancy between the Schilling test values and the serum levels of vitamin B_{12} still remains. This suggests that the cause of the discrepancy between the Schilling test values and the serum levels of vitamin B_{12} is not necessarily that of a methodological failure. There might be additional reason for the vitamin B_{12} deficiency in some tapeworm carriers other than the impaired absorption.

Large metamyelocytes as a sign of vitamin B_{12} deficiency

The appearance of large metamyelocytes in the bone marrow has been reported as

The serum level of vitamin B_{12} was normal in 82 cases In the bone marrow were found

fully developed megaloblasts in 1 case (1 per cent)
megaloblastic features in 4 cases (5 per cent) and
no signs of megaloblasts in 77 cases (94 per cent)

There is a significant difference between percentages of cases with megaloblasts in the first and the last group ($P < 0.1$ per cent)

A comparison made of the Schilling test values with the occurrence of the megaloblasts gave the following results

The 74 cases with pathological Schilling test values had in their bone marrow

fully developed megaloblasts in 26 cases (35 per cent)
megaloblastic features in 9 cases (12 per cent) and
no signs of megaloblasts in 39 cases (53 per cent)

Among the 39 cases with borderline values in the Schilling test there were in the bone marrow

fully developed megaloblasts in 3 cases (8 per cent)
megaloblastic features in 1 case (3 per cent) and
no signs of megaloblasts in 35 cases (89 per cent)

71 tapeworm carriers had normal Schilling test values In their bone marrow they had

fully developed megaloblasts in no case (0 per cent)
megaloblastic features in 4 cases (6 per cent) and
no signs of megaloblasts in 67 cases (94 per cent)

In this comparison also the difference between the percentage of the megaloblasts present in the first and that in the last group is significant ($P < 0.1$ per cent)

The Schilling test and the serum assay of vitamin B_{12} seem to be quite equivalent and rather reliable methods for measurement the vitamin B_{12} deficiency in tapeworm carriers, when evaluated with the occurrence of the megaloblasts in the same cases. None of them gave a perfect correlation with the occurrence of megaloblasts, and accordingly the results of the Schillling test were combined with the serum levels of vitamin B_{12}, and a comparison made with the megaloblast phenomenon.

Both the Schilling test value and the serum level of vitamin B_{12} were pathological in 41 patients, megaloblastic erythropoiesis was fully developed in 49 per cent (20 cases) and megaloblastic features in 15 per cent Both the Schilling test and the serum level of vitamin B_{12} were normal in 43 cases, in which there were no signs of megaloblasts. Needless to say the difference in the percentages of the megaloblastic cases in these groups is significant ($P < 0.1$ per cent)

The Schilling test value was on the borderline in 99 cases, for whom the numbers of large metamyelocytes were

pathological in 6 cases (5 per cent)
on the borderline in 6 cases (5 per cent) and
normal in 87 cases (70 per cent)

71 tapeworm carriers had normal Schilling test values, with numbers of large metamyelocytes which were

pathological in 4 cases (6 per cent)
on the borderline in 5 cases (per cent) and
normal in 52 cases (73 per cent)

The numbers of the pathological values in the first and the last group showed significant differences ($P < 0.1$ per cent)

As can be seen, the comparison of the number counts of large metamyelocytes with the results of the Schilling test, and the serum assay of vitamin B_{12}, gave similar results, even with similar percentages, the increased number of large metamyelocytes is dependent upon the disturbed vitamin B_{12} metabolism, as indicated by low Schilling test values and low serum levels of vitamin B_{12}.

A comparison of the large metamyelocytes in the bone marrow with the occurrence of the megaloblasts gave the following results

Megaloblastic erythropoiesis was fully developed in 29 cases, which all had pathologically increased numbers of large metamyelocytes.

Signs of incipient megaloblastic change of the erythropoiesis were seen in 14 cases, the number of large metamyelocytes was

pathological in 8 cases (57 per cent), and
on the borderline in 6 cases (43 per cent)

No signs of megaloblasts in the erythropoiesis were found in 141 cases, in which the numbers of large metamyelocytes were

pathological in 5 cases (3 per cent)
on the borderline in 5 cases (8 per cent) and
normal in cases (79 per cent)

From the data presented above it appears that the earliest megaloblastic features in the erythropoiesis were found when the number of large metamyelocytes was increased. In the presence of the fully developed megaloblastic erythropoiesis, in every case more than one half of the metamyelocytes exceeded 14 μ in diameter. The increase in the large metamyelocytes indeed seems to be related to the vitamin B_{12} deficiency.

Schilling test, serum level of vitamin B_{12}, large metamyelocytes, and megaloblasts

The interrelations of the Schilling test values, the serum levels of vitamin B_{12},

occurring at an earlier phase of vitamin B_{12} deficiency than the megaloblastic change of the erythropoiesis. Only a limited number of series have been subjected to measurement of the diameters of the metamyelocytes and to comparison of the proportion of large forms with the megaloblast phenomenon. It could be expected that the proportion of large metamyelocytes would give as exact a morphological measurement of the vitamin B_{12} deficiency as the megaloblasts. Thus in the present series the proportions of the large metamyelocytes were compared with the occurrence of megaloblasts and *other signs of vitamin B_{12} deficiency*.

That the increased proportion of large metamyelocytes in tapeworm carriers is in fact brought about by the vitamin B_{12} deficiency is strongly suggested by their *absence in all those cases examined two* months after worm expulsion and Schilling test, irrespective of the result of the worm cure. Another suggestion in the same direction is concerned with the comparison of the large metamyelocytes with the serum levels of vitamin B_{12}. In the whole series of 184 patients with tapeworm pernicious anaemia and tapeworm carriers, the serum level of vitamin B_{12} was pathological in 71 cases. The number *of large metamyelocytes* was

pathological in 29 cases (41 per cent)
on the borderline in 11 cases (15 per cent) and
normal in 31 cases (44 per cent)

In the 31 cases with borderline values of vitamin B_{12} in the serum the number of the large metamyelocytes was

pathological in 8 cases (26 per cent)
on the borderline in 4 cases (13 per cent) and
normal in 9 cases (6 per cent)

The 82 cases with normal serum levels of vitamin B_{12} had large metamyelocytes which were

pathological in 5 cases (6 per cent)
on the borderline in 16 cases (20 per cent) and
normal in 61 cases (74 per cent)

The percentages of cases with pathological numbers of large metamyelocytes in the first and the last group had significant difference ($P < 0.1$ per cent).

When the results of the Schilling tests were taken against the number of large metamyelocytes, the following comparative figures were obtained.

The Schilling test values were pathological in 74 cases; the numbers of large metamyelocytes were here

pathological in 3 cases (43 per cent),
on the borderline in 0 cases (4 per cent) and
normal in 32 cases (43 per cent)

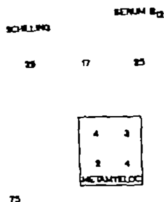


Fig. a.

Diagram showing the distributions of the pathological values of the metamyelocytes, the serum assays of vitamin B_{12}, and the Schilling tests, and their various combinations, b d in the tapeworm carriers. The area is in relation to the number of cases. The numbers of cases with various combinations are expressed by numerals.

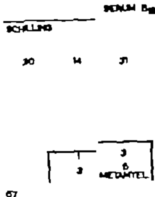


Fig.

Diagram showing b l t y distributions of the various combinations of the pathological values of the metamyelocytes, the serum assays of vitamin B_{12}, and the Schilling tests in the tapeworm carriers. The area is in relation to the number of cases. The numbers of cases with various combinations are expressed by numerals.

marrow and peripheral blood count The peripheral blood count before the worm expulsion was compared with that taken subsequently It was accepted that an improvement had occurred in the cases whose red blood cell count had increased by more than 500,000 at the re-examination. Two further cases, whose red blood cell count had increased by 200,000 t 300,000 per cub.mm., and a reduction in the mean corpuscular haemoglobin from 31 to 29 wer included in the same group. The changes in the peripheral blood count in these cases, along with one further case whose granulocyte count had risen from 1,800 t 2,900, are compared with the bone marrow findings in Table 5. It should be noted that the granulocyte counts had increased at least by 500 per cub. mm. Changes in the peripheral blood count were recorded in 11 of these 42 tapeworm carriers. The four cases with some megaloblastic features in their erythropoiesis had all shown higher red blood cell counts after worm cure. Most of the cases with pathological numbers of large metamyelocytes showed changes in their peripheral blood count. Of the 25 cases with a normal proportion of large metamyelocytes, three had changes in the peripheral blood count, two of them (cases 59 and 135) mainly what was a granulocytopenia. The proportion of the cases with changes in the peripheral blood count in those with megaloblastic features, and those with pathological numbers of large meta-

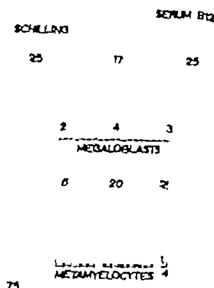


Fig 9.

Diagram showing the distributions of the megaloblastic erythropoiesis, and the pathological values of the metamyelocytes, the serum assays of vitamin B_{12}, and the Schilling tests, and their various combinations in the present series as a whole. The area is in relation to the number of cases. The numbers of cases with various combinations are expressed by numerals.

the large metamyelocytes, and the megaloblasts in the present series as a whole have been summarized in Figure 9, in which are illustrated the distributions of the combinations of pathological values. It is apparent that the pathological large metamyelocytes completely cover the cases with megaloblasts. Most of these cases were in the common area of pathological Schilling test values and serum levels of vitamin B_{12}. The only discrepancy is the large number of pathological serum levels of vitamin B_{12} outside the pathological Schilling test values.

When the tapeworm carriers alone — excluding the 29 cases of tapeworm pernicious anaemia with megaloblastic erythropoiesis — were taken into account, the correlations of the results obtained by the various methods were not as clear as in the material as a whole. The distribution of the combinations of pathological values in the Schilling tests, the serum assays of vitamin B_{12}, and the measurements of the large metamyelocytes have been illustrated in Figure 10. For the sake of comparison there is presented in Figure 11 a hypothetical distribution of the combinations calculated from the separate results, assuming that no causal relationship exists between the results. The figures are surprisingly alike. The only observed number that differs to an almost significant extent ($P < 5$ per cent) from the arbitrary one, is the combination of pathological values obtained by means of all three methods used.

Peripheral blood count

As regards patients suffering from tapeworm pernicious anaemia, the criterion accepted was the occurrence of a fully developed megaloblastic erythropoiesis. Distinct changes in the peripheral blood count were encountered in all of them: a hyperchromic macrocytic anaemia, often granulocytopenia. However the most interesting finding in this group was that all the patients with fully developed megaloblastic erythropoiesis had a haemoglobin figure of less than 10 g per 100 ml.

In the group of tapeworm carriers after a successful worm cure, 42 cases could be re-examined in respect of bone

Table 6. Data on 17 tapeworm carriers with signs of incipient tapeworm pernicious anaemia.

Case number	Age	Sex	Month	Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes	Acid A	Schilling	Serum B_{12}	Large ...	Megaloblastic marrow
29	76	f	VII	9.6	2.5	30	120	32	38	2.4	3,500	0.28	11.3	140	79	+
31	66	m	VIII	10.1	3.2	33	104	30	32	0.8	7,000	0.55	1.0	95	36	+
			X	*14.35*	*4.1*	*45*	*110*	*31*	*35*		*9,700*	*0.2*		*> 1000*	*21*	—
32	63	f	VII	10.1	2.95	32	108	32	34	0.5	2,500	0.26	12.6	15	59	+
39	72	f	VI	11.0	3.25	36	112	30	34	5.8	7,500	1.4	4.6	45	79	+
			VIII	*12.9*	*4.2*	*40*	*96*	*32*	*31*		*6,200*			*> 1000*	*11*	—
41	68	m	VIII	11.2	3.7	39	106	29	30	1.4	6,500	0.9	0.2	220	33	+
42	47	m	VI	11.2	4.0	38	95	30	28	0.1	7,000	0.12	0	60	61	—
45	17	f	VIII	11.35	3.7	38.5	104	29	31	1.7	5,600	0.4	7	240	69	+
			X	*13.7*	*3.8*	*42*	*108*	*32*	*35*	*1.6*	*3,700*	*2.8*		*200*	*17*	—
53	37	m	II	11.7	3.45	37	108	32	34	1.0	6,500	0.05	0	< 10	53	+
			VI	*14.75*	*5.25*	*48*	*92*	*30*	*28*	*0.3*	*6,400*	*0.4*		*190*	*13*	—
63	60	f	IX	12.2	3.7	42	114	29	33	1.1	3,700	0.81	15.0	11	35	+
			VII	*11.9*	*4.0*	*38.5*	*96*	*31*	*30*	*0.6*	*3,200*			*> 1000*	*16*	—
72	72	f	III	12.3	3.4	40	118	31	36	1.5	3,100	0.69	10.0	< 10	42	+
			VI	*15.0*	*4.85*	*45.5*	*94*	*33*	*31*	*0.9*	*3,600*	*0.17*		*140*	*27*	—[1]
78	77	f	IV	12.6	3.4	40	118	31	37	3.8	2,400	0.06	0.7	11	80	+
			VI	*15.0*	*5.0*	*46.5*	*94*	*32*	*30*	*1.1*	*2,600*			*> 1000*	*29*	—
89	74	f	VII	12.9	4.6	44	96	29	28	0.7	5,100	0.37	2.4	350	57	+
92	40	f	IV	12.9	4.18	42	100	31	31	0.8	3,100	2.5	8.0	*11*	*67*	—
			VII	*13.7*	*5.9*	*47*	*80*	*29*	*23*	*0.8*	*5,400*			*500*	*8*	—
111	39	m	VII	13.5	4.2	40.8	97	33	32	1.0	2,800	0.15	1.2	250	72	+
115	46	m	III	13.5	4.25	42	99	32	32	0.8	4,100	0.8	8.2	125	50	—[1]
			VI	*13.7*	*4.75*	*46.5*	*98*	*29*	*29*	*0.5*	*4,600*	*0.5*		*> 1000*	*7*	—
119	26	m	IV	13.7	4.12	43	108	30	33	1.0	7,400	0.76	1.0	< 10	45	+
			VII	*12.2*	*4.1*	*40.5*	*99*	*30*	*30*	*0.8*	*7,700*			*20*	*6*	—[1]
128	27	m	II	13.9	4.25	41	97	34	33	2.0	3,800	0.82	1.3	16	40	+
			VI	*15.2*	*5.4*	*48.5*	*90*	*31*	*28*	*0.7*	*5,800*			*35*	*6*	—

[1] Tapeworm eggs still found.

Table 5. Change in the peripheral blood count compared with the changes in bone marrow of 42 tapeworm carriers in whom the haematological examination could be carried out before and after successful worm cure.

Bone marrow finding	Whole group	Number of cases with changes in the red blood cell count	Number of cases with changes in the granulocyte count	Total
Megaloblastic features	4	4	2	4 (100 per cent)
Pathological number of large metamyelocytes	5	4	2	4 (80 per cent)
Borderline number of large metamyelocytes	13	4	2	6 (31 per cent)
Normal metamyelocytes	25	2	2	3 (12 per cent)
Total	42	10	6	11 (25 per cent)

myelocytes differ significantly from the group with normal metamyelocytes (P < 1 per cent) The groups with borderline and normal numbers of large metamyelocytes did not differ significantly in their peripheral blood changes

The change seen in the peripheral blood count does not occur before increase in large metamyelocytes in the tapeworm carriers. On the other hand, as all the cases with fully developed megaloblastic erythropoiesis had distinct anaemia, it seems that the early changes in the peripheral blood count occur simultaneously with the early changes in the bone marrow before any signs of megaloblasts may be found

As it was not possible to re-examine all the cases, and even less after a *successful* worm expulsion the comparison given above does not include the total of patients which could be regarded as incipient tapeworm pernicious anaemia cases. Accordingly data concerning the 14 tapeworm carriers in whom incipient signs of megaloblastic change of their erythropoiesis had been detected are given in Table 6 Three cases with pathologically increased numbers of large metamyelocytes have been added since they also had other indications of incipient tapeworm pernicious anaemia. The group thus consisted of 17 cases, most of whom had pathological Schilling test and serum B_{12} values. In some cases slight anaemia was present. After a successful worm expulsion, the bone marrow changes had disappeared and the haemoglobin value, the red blood cell count, the mean corpuscular haemoglobin, and the granulocyte count had improved There seems to be justification for considering these 17 cases (11 per cent of the tapeworm carriers in the present series) as patients with incipient tapeworm pernicious anaemia. This proportion is very high in comparison with the occurrence of fully developed tapeworm pernicious anaemia (2 per cent according to Nyberg 1960)

Neurological disturbance

The neurological disturbance in the present series was compared with the haematological findings, the serum level of vitamin B_{12}, and the Schilling test values. As the signs of the neurological disturbance are essentially subjective, the only cases taken for this comparison

Table 6. Data on 17 tapeworm carriers with signs of incipient tapeworm pernicious anaemia.

Case number	Age	Sex	Month	Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes	[illegible] A	[illegible]	Serum B_{12}	[illegible]	[illegible]
29	76	f	VII	9.6	2.5	30	120	32	38	2.4	3,300	0.28	11.5	140	79	+
31	66	m	VIII	10.1	3.2	33	104	30	32	0.8	7,000	0.55	1.0	95	56	+
			X	*14.35*	*4.1*	*45*	*110*	*31*	*35*		*9,700*	*0.2*		> *1000*	*21*	—
32	63	f	VII	10.1	2.95	32	108	32	34	0.5	2,500	0.26	12.6	15	59	+
38	72	f	VI	11.0	3.25	36	112	30	34	5.8	7,500	1.4	4.6	45	78	+
			VIII	*12.9*	*4.2*	*40*	*96*	*32*	*31*		*6,200*			> *1000*	11	—
41	68	m	VIII	11.2	3.7	39	106	29	30	1.4	6,300	0.9	0.2	220	53	+
42	47	m	VI	11.2	4.0	38	95	30	28	0.1	7,000	0.12	0	60	61	—
43	17	f	VIII	11.35	3.7	38.5	104	29	31	1.7	3,600	0.4	7	240	60	+
			X	*13.7*	*3.9*	*42*	*108*	*32*	*35*	*1.6*	*5,700*	*2.0*		*200*	*17*	—
53	57	m	II	11.7	3.45	37	108	32	34	1.0	6,500	0.09	0	< 10	55	+
			VI	*14.75*	*5.25*	*46*	*92*	*30*	*28*	*0.5*	*6,400*	*0.4*		*150*	*13*	—
65	60	f	IV	12.2	3.7	42	114	29	33	1.1	3,700	0.91	15.0	11	53	+
			VII	*11.9*	*4.0*	*38.5*	*96*	*31*	*30*	*0.6*	*3,200*			> *1000*	*16*	—[1]
72	72	f	III	12.5	3.4	40	118	31	36	1.5	3,100	0.09	10.0	< 10	42	+
			VI	*15.0*	*4.85*	*45.5*	*94*	*33*	*31*	*0.9*	*3,600*	*0.17*		*140*	*27*	—[1]
78	77	f	IV	12.6	3.4	40	118	31	37	3.8	2,400	0.06	0.7	11	80	+
			VI	*15.0*	*5.0*	*46.5*	*94*	*32*	*30*	*1.1*	*2,600*			> *1000*	*25*	—
89	74	f	VII	12.9	4.6	44	96	29	28	0.7	3,100	0.37	2.4	350	57	+
97	40	f	IV	12.9	4.18	42	100	31	31	0.8	3,100	2.5	8.0	11	67	—
			VII	*13.7*	*5.9*	*47*	*80*	*29*	*23*	*0.8*	*3,400*			*500*	*6*	—
111	59	m	VII	13.3	4.2	40.8	97	33	32	1.0	2,800	0.15	1.2	250	72	+
113	46	m	III	13.3	4.25	42	99	32	32	0.8	4,100	0.8	8.2	123	30	—?
			VI	*13.7*	*4.75*	*46.5*	*98*	*29*	*29*	*0.9*	*4,600*	*0.9*		> *1000*	*7*	—
119	26	m	IV	13.7	4.12	43	*100*	*30*	*33*	*1.0*	7,400	0.76	1.0	< 10	45	+
			VII	*12.2*	*4.1*	*40.5*	*99*	*30*	*30*	*0.8*	*7,700*			*20*	*6*	—
120	37	m	II	13.9	4.23	41	97	34	33	2.0	3,900	0.82	1.5	16	40	+
			VI	*15.2*	*5.4*	*48.5*	*90*	*31*	*28*	*0.7*	*5,800*			*35*	*6*	—

[1] tapeworm eggs still found.

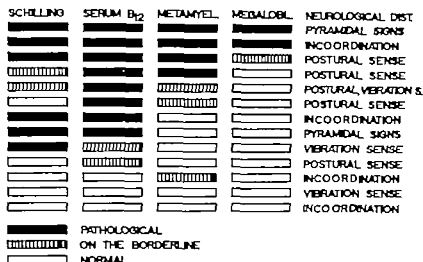


Fig 12.

Diagram showing the neurological disturbance compared with other signs of vitamin B_{12} deficiency in the 13 cases which showed clear improvement two *months after a successful* worm cure.

were those in which the improvement at the follow up examination was obvious.

In the whole of the present series, the neurological disturbance was found to have been clearly improved in 13 cases after a successful worm cure. In these cases *the neurological disturbance* has been compared in Figure 12 with the Schilling test value, the serum level of vitamin B_{12}, *the number of large* metamyelocytes and the megaloblast phenomenon. In the last two cases in the figure the results of the other methods did not support an assumption of vitamin B_{12} deficiency As for the remaining eleven cases, four had neither signs of megaloblasts, nor an increased number of large metamyelocytes.

Azur A resin test meal

The results obtained from the azur A resin test meal in the present series were *compared with the serum level of vitamin* B_{12}. In the tapeworm carriers, the numbers of test values suggesting gastric achlorhydria did not change significantly with the serum level of vitamin B_{12}.

DISCUSSION OF THE INTERRELATIONS OF THE INDICATIONS OF VITAMIN B_{12} DEFICIENCY

The intensive studies on the pathogenesis of tapeworm pernicious anaemia reviewed earlier suggests that it is a deficiency state of vitamin B_{12} similar to Addisonian pernicious anaemia. On the basis of the recent investigations on vitamin B_{12} metabolism in tapeworm carriers, v Bonsdorff concluded that practically all of the carriers of the fish tapeworm show disturbed absorption of vitamin B_{12} (v Bonsdorff 1959, 1961 Bonsdorff et al. 959) Accordingly v Bonsdorff further concluded that no sharp distinction could be made between anaemic and non-anaemic tapeworm carriers. Further to this, Pedersen et al (9*7) found among elderly achlorhydric patients with a mild anaemia some cases with partial megaloblastic erythropoiesis. They assumed that pernicious anaemia does not obey the law »all or nothing» but that cases in an earlier stage of the deficiency may be detected.

In the present series of patients, pathological Schilling test values and serum levels of vitamin B_{12} were found to be the most common signs of disturbed vitamin B_{12} metabolism in tapeworm carriers. In the majority of instances they preceded the occurrence of other signs. The number of the pathological serum levels of vitamin B_{12} had fallen significantly two months subsequently to the Schilling test and a successful worm expulsion. In those cases in which the worm cure had proved unsuccessful, the vitamin B_{12} dose (1 mg parenterally) given in the Schilling test was unable to maintain the number of the pathological serum levels of vitamin B_{12} at a significantly lower value at the re-examination than before the test and worm cure. The result suggests the causal relationship of the fish tapeworm and vitamin B_{12} deficiency which is in agreement with the findings of earlier investigations.

The Schilling test values and the serum levels of vitamin B_{12} provided a close correlation with the megaloblast phenomenon — a generally accepted indication of vitamin B_{12} deficiency. Accordingly both of these methods seemed to give clinically useful results.

In the haematopoesis, the earliest change in the present series was observed to occur in the myelopoesis. If the percentage of large metamyelocytes exceeding 14 μ in diameter was less than 30, no signs of megaloblasts were found. In six cases out of 31 (8 per cent) indications of an incipient megaloblastic maturation disturbance were found when the large metamyelocytes amounted to 30 to 49 per cent. Among the 13 tapeworm

carriers with large metamyelocytes of 50 per cent or more, eight (62 per cent) showed incipient signs of megaloblastic maturation disturbance in the erythropoiesis. All of the 29 tapeworm pernicious anaemia patients with typical megaloblastic erythropoiesis had large metamyelocytes amounting to 50 per cent or more. The result of the re-examination two months after the Schilling test and worm expulsion provides a strong suggestion that the increase in number of the large metamyelocytes is due to a vitamin B_{12} deficiency. After the parenterally administered dose of 1 mg vitamin B_{12}, the number of large metamyelocytes two months later was normal in every case, irrespective of the success or failure of the worm expulsion. The megaloblasts and even the incipient megaloblastic features disappeared in the same way after the Schilling test, independent of the worm expulsion. The fact that the earliest indication of megaloblastic maturation disturbance of the erythroid cells in the present series of patients were seen only when the number of the large metamyelocytes was increased is in agreement with the observations made by Bastrup-Madsen (1952, 1954, 1956), Jewesbury (1954), Munch-Petersen (1955), Kristensen and Ohlsen (1956), Hansen and Paulsen (1957), Pedersen et al. (1957), Kristensen and Gormsen (1958), Kristensen et al. (1958), Heinivaara and Kaipainen (1959, 1960), Hansen (1960) and Swedberg (1960).

The changes in the peripheral blood count were seen in only a limited number of tapeworm carriers. Only 12 of the 42 tapeworm carriers who could be re-examined after a successful worm expulsion showed a change which could be attributable to tapeworm infestation. The most pronounced changes observed in the present series seemed to be those in the red blood cells. However, the change in the granulocyte count might in some instances have been masked by intercurrent infections. In general, the change in the myeloid series of the bone marrow in respect of which the occurrence of large metamyelocytes was evaluated in the present series, appeared to occur in the same phase of vitamin B_{12} deficiency as the earliest changes in the peripheral blood count. When the first signs of megaloblastic change were detected in the erythropoiesis, many tapeworm carriers had developed a slight anaemia. The anaemia was clearly distinguishable in all cases in which the megaloblastic erythropoiesis was well developed.

Neurological disturbances which could be proved as being caused by the tapeworm infestation were seen in only 13 cases of the whole present series. The relatively short time lapse between the examinations, coupled with rather unsuccessful worm cures, diminished the number in this group. Eleven of the cases with neurological disturbance also evinced other signs of vitamin B_{12} deficiency. The results suggest that in the main neurological disturbance in tapeworm carriers is due to vitamin B_{12} deficiency. Seven of the cases with neurological disturbance had haematological changes. As the number of cases is limited, no conclusion may be drawn on the relative incidences of neurological and haematological disturbances in tapeworm carriers. Nevertheless, the number is adequate to show that neurological signs

of vitamin B_{12} deficiency may occur earlier than haematological signs in tapeworm carriers.

The problem of the incidence of tapeworm pernicious anaemia among carriers of the fish tapeworm seems largely to be linked to the criteria used. In the present series of patients an ppreciably large group of tapeworm carriers — 11 per cent — was found with indications of incipient disturbance in the haematopoiesis. Only one of them had been admitted to hospital by reason of the haematological changes. Most of them were not properly anaemic, but showed improvement in the peripheral blood count after worm cure and the Schilling test. As the change was observed in bone marrow and not in the peripheral blood count at the first examination, in earlier studies many such cases have been accepted without further comment in the group of non-anaemic tapeworm carriers. On the other hand, many of these cases may with good reason be included in the number of tapeworm pernicious anaemia cases. The incidence of the latter should then be within the range of 0 to 15 per cent of tapeworm carriers. In Nyberg's (1960) series, two per cent of tapeworm carriers had tapeworm pernicious anaemia. It thus appears essential to report exactly which criteria are used if any statement is to be made on the incidence of tapeworm pernicious anaemia among tapeworm carriers.

Whatever criteria may be used in calculations on the occurrence of tape worm pernicious anaemia among the carriers of fish tapeworm, the fact still remains that only a minority of the tapeworm carriers develop megaloblastic anaemia. v Bonsdorff et al. (1959) explained that the relatively low incidence of manifest B_{12} deficiency is mainly due to the B_{12} pool in the body being large. Under certain circumstances it might be exhausted in tapeworm carriers. The circumstances instanced by v Bonsdorff et al. further included that of the worm being situated high up in the intestine, the large amount of the worm present, the decreased secretion of the intrinsic factor and the inadequate supply of vitamin B_{12} in the food. They also bore in mind the possibly increased need of the host organism for vitamin B_{12}.

Earlier studies make it evident that the role of the parasite is essential in the development of the vitamin B_{12} deficiency leading to megaloblastic tapeworm pernicious anaemia in some carriers of *Diphyllobothrium latum*. The average amount of vitamin B_{12} in the daily food (Estren et al. 1958) ppears to approximate to the daily need for it (Gräsbeck 1960). The fish tapeworm takes a considerable amount of the vitamin B_{12} available — an average of 43.9 per cent in the series of Nyberg (1958). The average tapeworm carrier thus seems to live at a minimum of existence in his vitamin B_{12} metabolism. Even minor additional factors may accord ingly disturb the labile balance, and bring about a manifestation of the vitamin B_{12} deficiency state.

The decreased intrinsic factor activity has been evaluated by many authors as of extreme importance in the development of the vitamin B_{12} deficiency as was indicated in the earlier review. In the present investigation, it was not possible to perform new Schilling tests at the

carriers with large metamyelocytes of 50 per cent or more, eight (62 per cent) showed incipient signs of megaloblastic maturation disturbance in the erythropoiesis. All of the 29 tapeworm pernicious anaemia patients with typical megaloblastic erythropoiesis had large metamyelocytes amounting to 50 per cent or more. The result of the re-examination two months after the Schilling test and worm expulsion provides a strong suggestion that the increase in number of the large metamyelocytes is due to a vitamin B_{12} deficiency. After the parenterally administered dose of 1 mg vitamin B_{12}, the number of large metamyelocytes two months later was normal in every case, irrespective of the success or failure of the worm expulsion. The megaloblasts and even the incipient megaloblastic features disappeared in the same way after the Schilling test, independent of the worm expulsion. The fact that the earliest indication of megaloblastic maturation disturbance of the erythroid cells in the present series of patients were seen only when the number of the large metamyelocytes was increased is in agreement with the observations made by Bastrup-Madsen (1952, 1954, 1956), Jewesbury (1954), Munch-Petersen (1955), Kristensen and Ohlsen (1956), Hansen and Paulsen (1957), Pedersen et al. (1957), Kristensen and Gormsen (1958), Kristensen et al. (1958), Heinivaara and Kaipainen (1959, 1960), Hansen (1960) and Swedberg (1960).

The changes in the peripheral blood count were seen in only a limited number of tapeworm carriers. Only 12 of the 42 tapeworm carriers who could be re-examined after a successful worm expulsion showed a change which could be attributable to tapeworm infestation. The most pronounced changes observed in the present series seemed to be those in the red blood cells. However, the change in the granulocyte count might in some instances have been masked by intercurrent infections. In general, the change in the myeloid series of the bone marrow in respect of which the occurrence of large metamyelocytes was evaluated in the present series, appeared to occur in the same phase of vitamin B_{12} deficiency as the earliest changes in the peripheral blood count. When the first signs of megaloblastic change were detected in the erythropoiesis, many tapeworm carriers had developed a slight anaemia. The anaemia was clearly distinguishable in all cases in which the megaloblastic erythropoiesis was well developed.

Neurological disturbances which could be proved as being caused by the tapeworm infestation were seen in only 15 cases of the whole present series. The relatively short time lapse between the examinations, coupled with rather unsuccessful worm cures, diminished the number in this group. Eleven of the cases with neurological disturbance also evinced other signs of vitamin B_{12} deficiency. The results suggest that in the main neurological disturbance in tapeworm carriers is due to vitamin B_{12} deficiency. Seven of the cases with neurological disturbance had haematological changes. As the number of cases is limited, no conclusion may be drawn on the relative incidences of neurological and haematological disturbances in tapeworm carriers. Nevertheless, the number is adequate to show that neurological signs

normal Schilling test values had pathological serum levels of vitamin B_{12}. Some of these cases also showed bone marrow changes indicating vitamin B_{12} deficiency In these cases there were accordingly indications of vitamin B_{12} deficiency despite a vitamin B_{12} absorption that should have been able to cover the normal need if the dietary supply of vitamin B_{12} had been adequate. The only possible explanation for these findings seem to be that of assuming an insufficient dietary supply of vitamin B_{12}, in comparison with the need, to be of essential importance in the development of the vitamin B_{12} deficiency in many carriers of fish tape worm.

follow up examination. Thus no data are available as regards the intrinsic factor activity in the present series. However the older tapeworm carriers had pathological Schilling test values to an almost significant extent more often than had the young ones. Most of the tapeworm pernicious anaemia cases in the present series were above 60 years of age. This may suggest a decreased intrinsic factor activity in many cases of tapeworm pernicious anaemia.

The gastric achlorhydria in the tapeworm pernicious anaemia cases may be primary or caused by an inhibitory effect of the worm, or be due to the vitamin B_{12} deficiency It appears from the findings of Siurala (1954, 1956) that the last mentioned is the most probable. In the present series, there was found no correlation between the azur A resin test meal values and the serum levels of vitamin B_{12}. All of the ten cases with values which suggested gastric achlorhydria subjected to re-examination after a successful worm expulsion had the azur A resin test values unchanged A period of two months may be too short for improvement of the gastric secretion. The number of the cases re examined was also limited. But both of these comparisons gave similar results the present series of patients gave no proof that gastric achlorhydria in tapeworm carriers is caused by vitamin B_{12} deficiency Nevertheless, it should be mentioned that the method utilized was rather crude in nature

The increased need of the host organism for vitamin B_{12} may be taken into consideration under certain circumstances (v Bonsdorff et al. 1959) In the present series, none of the tapeworm pernicious anaemia patients was pregnant, or thyrotoxic. Other possible causes of an increased need for vitamin B_{12} are not so clear The seasonal variations in the need for vitamin B_{12} might constitute an especially interesting object of study

The deficiency of animal protein in the diet may bring about an increase in the incidence of tapeworm pernicious anaemia among tapeworm carriers (Tötterman 1944) During studies on the pathogenesis of tapeworm pernicious anaemia, v Bonsdorff and Gordin (1951) assumed a deficient dietary supply of vitamin B_{12} to be an important etiological factor in many of their cases of tapeworm pernicious anaemia. Landström (1929) observed seasonal variations in the incidence of tapeworm pernicious anaemia In the present series, seasonal variations occurred in the serum levels of vitamin B_{12} in tapeworm carriers. No earlier investigations concerned with the seasonal variations in serum levels of vitamin B_{12} in Finns with or without fish tapeworm are available. Furthermore, no information is available on the amounts of the mean daily supply of vitamin B_{12} in the Finnish diet during various seasons. There exists no logical reason for the assumption of seasonal variations in the absorption of vitamin B_{12} with the intrinsic factor mechanism. There is probably no more reason to assume that the situation of the worm — high up, or lower down in the intestine — or the amount of the worm would undergo seasonal variations. In the present series, no seasonal variation occurred in the Schilling test values. Comparison of the Schilling test values with the serum levels of vitamin B_{12} gave the result that some tapeworm carriers with

SUMMARY

A review is given of the publications concerned with tapeworm pernicious anaemia, and a brief description provided of some historical aspects. Attention has been concentrated on the behaviour of tapeworm pernicious anaemia as a manifest deficiency state of vitamin B_{12}, similar to Addisonian pernicious anaemia. Studies made to determine why only a small minority of the carriers of the fish tapeworm develop tapeworm pernicious anaemia are also reviewed.

The purpose of the present study was that of simultaneous investigation, with a large series of patients, of several components of the vitamin B_{12} metabolism in tapeworm carriers, and in patients suffering from tapeworm pernicious anaemia, in order to determine the interrelations of the indications of vitamin B_{12} deficiency obtained by the various methods available.

The present series of patients consisted of 55 carriers of the fish tapeworm under treatment for various ailments in the medical wards of the Central Hospital of Northern Karelia (in 1959). The series further included 29 patients suffering from tapeworm pernicious anaemia. The criterion adopted for the last mentioned was the occurrence of a typical megaloblastic erythropoiesis in the sternal marrow aspirate.

The mean age of the patients with tapeworm pernicious anaemia in the present series was higher than that of the other tapeworm carriers. More than one half of the first-mentioned were 60 or more years of age.

During the course of the present study the incidence of tapeworm carriers among the patients of the Central Hospital of the Northern Karelia amounted to 15.6 per cent.

The diagnosis of tapeworm pernicious anaemia was based upon the typical megaloblasts in the bone marrow not upon the peripheral blood count. These indicated a hyperchromic macrocytic anaemia t a haemoglobin level of less than 10 g per 100 ml, furthermore a granulocytopenia was seen. As regards the nonanaemic carriers as a group, no change in the haemoglobin, or the number of the red blood cells was seen after the worm cure. In the main, the number of granulocytes showed a rising tendency after the worm cure.

In the azur A resin test meal, most of the tapeworm carriers showed values which suggested the presence of free hydrochloric acid in their stomachs. In most of the tapeworm pernicious anaemia patients, the results of the same tests suggested gastric achlorhydria. After successful expulsion of the worm had been

CONCLUSIONS

Various stages of vitamin B_{12} deficiency are to be found in carriers of the fish tapeworm Many tapeworm carriers display no detectable indication of vitamin B_{12} deficiency The most common and most probably the earliest signs of disturbed vitamin B_{12} metabolism in tapeworm carriers are deduced from Schilling test values and serum levels of vitamin B_{12} below the normal range. More rarely tapeworm carriers develop myelopoiesis disturbance in the form of an increased number of large metamyelocytes in the bone marrow aspirate It is likely that this occurs at a stage later than that of the disturbances in the absorption and in the serum level of vitamin B_{12}. The changes appear to occur latest in the erythropoiesis The first features of the megaloblastic disturbance in the maturation of the erythroid cells are found only when there is at least a moderate increase in the number of large metamyelocytes. The appearance of the typical megaloblasts is connected with the dominance of large forms of the metamyelocytes

The changes in the peripheral blood count appear at the same stage of vitamin B_{12} deficiency as the change in the metamyelocytes but earlier than the first megaloblastic features can be detected

In some cases, neurological disturbance due to a vitamin B_{12} deficiency in tapeworm carriers occurs earlier than does the haematological change.

If any statements are to be made at all on the incidence of tapeworm pernicious anaemia among the carriers of fish tapeworm there is every justification for the provision of exact information on the criteria used for the diagnosis. There exists a large group of transitional forms which may with just as good reason dependent on the criteria used, be included in the tapeworm pernicious anaemia group or in non anemic tapeworm carriers. As these appear to be five times as common as the fully developed megaloblastic forms they may confuse all the calculations made on the incidence of tapeworm pernicious anaemia among the carriers of fish tapeworm.

Apart from the parasite, which competes with the host organism for the vitamin B_{12} available, additional factors seem to be present in many of the fish tapeworm carriers who develop a deficiency of vitamin B_{12}. Such factors might be the decreased intrinsic factor activity and the dietary supply of vitamin B_{12}, which is insufficient to meet the need

The large metamyelocytes were compared with the serum level of vitamin B_{12} and the megaloblasts. The increased number of large metamyelocytes was in correlation with the vitamin B_{12} deficiency as judged by the serum level of vitamin B_{12} and the megaloblast phenomenon. After the administration of vitamin B_{12} (Schilling test) the increased number of large metamyelocytes disappeared, irrespective of the result of the worm cure. The increase in the large metamyelocytes therefore seems to be related to the vitamin B_{12} deficiency. The number of large metamyelocytes increases before the appearance of indications of the megaloblastic change in the erythropoiesis.

In 11 tapeworm carriers, an improvement of the peripheral blood count was recorded after a successful worm expulsion. Eight of them had an increased number of large metamyelocytes (at least 90 per cent) and in four were observed incipient megaloblastic features in the erythropoiesis. The earliest changes in the bone marrow seem to appear simultaneously with the changes in the peripheral blood count. On the other hand, the anaemia is well developed (haemoglobin less than 10.0 g per 100 ml) when the erythropoiesis is typically megaloblastic.

Neurological disturbance was clearly improved after a successful worm cure as regards 13 cases, 11 of whom had evinced other signs of vitamin B_{12} deficiency. Seven cases had haematological disturbances. Thus the neurological signs of vitamin B_{12} deficiency in tapeworm carriers in some instances develop earlier than the haematological signs.

Azur A resin test meal values in the tapeworm carriers were not found to be in correlation with the serum levels of vitamin B_{12}.

Seventeen of the 155 tapeworm carriers were regarded as incipient cases of tapeworm pernicious anaemia. Fourteen of them showed megaloblastic features in the erythropoiesis, and all had an increased number of large metamyelocytes. The majority of them had low normal haemoglobin values and red blood cell counts; nevertheless, those had risen two months after worm cure. The mean corpuscular haemoglobin values, which were mostly high, had become normal after expulsion of the worm. The significance of these incipient cases is their relatively high incidence (11 per cent) as compared with the fully developed cases (2 per cent) of tapeworm pernicious anaemia. If studies are accordingly initiated on the incidence of tapeworm pernicious anaemia, the criteria accepted may have a decisive effect upon the results achieved.

effected, no return of free hydrochloric acid was to be observed

On the application of the Schilling test, with a test dose of 0 67 μg of ^{60}Co-labelled vitamin B_{12}, 31 per cent of the tapeworm carriers showed a urinary excretion of less than 5 (per cent) 23 per cent between 5 and 9 9 (per cent) and 46 per cent 10 (per cent) or more. Among the patients with tapeworm pernicious anaemia, the values were in respect of 90 per cent of the cases less than 5 (per cent) and in 10 per cent between 5 and 9 9 (per cent) A tendency towards lower values with advancing age was observed in the tapeworm carriers.

The serum levels of vitamin B_{12} determined by the utilization of *Euglena gracilis* as test organism were as regards the tapeworm carriers 100 $\mu\mu g$ per ml or less in 32 per cent 101 to 200 $\mu\mu g$ per ml in 16 per cent and more than 200 $\mu\mu g$ per ml in 52 per cent of cases. As for tapeworm pernicious anaemia, the corresponding figures were 76 21 and 3 per cent. Values below normal were obtained more often during the months of winter and spring than in the summer Two months after a successful worm cure had been effected the serum levels of vitamin B_{12} were less than 100 $\mu\mu g$ per ml in 5 per cent out of 42 tapeworm carriers and 101 to 200 in 11 per cent

Among the 155 tapeworm carriers, 14 showed features of incipient megaloblastic maturation disturbance in their erythropoiesis. In the re-examination two months subsequent to worm cure and Schilling test, all the megaloblastic features had disappeared, and in every case observed this was independent of the success of the worm cure

Metamyelocytes exceeding 14 μ in diameter were present to the extent of more than 50 per cent in all the cases of tapeworm pernicious anaemia in the present series. In the tapeworm carriers, the bone marrow showed large metamyelocytes to the extent of more than 50 per cent in 8 per cent of the cases, between 30 and 49 in 20 per cent and less than 30 in 72 per cent. Among 42 tapeworm carriers, two months subsequent to a successful worm expulsion, the number of large metamyelocytes was less than 30 per cent in each case.

A study was made of the interrelations of the signs of vitamin B_{12} deficiency obtainable by means of the various methods.

The Schilling test values and the serum levels of vitamin B_{12} were taken against the megaloblast phenomenon, which is the most reliable clinical proof of vitamin B_{12} deficiency Both the Schilling test values and the serum levels of vitamin B_{12} afforded a significant correlation with megaloblastic erythropoiesis. The correlation was almost perfect when the combined Schilling test and serum B_{12} values were compared with the megaloblasts. Thus it appears that in a combination they provide a rather reliable indication of the vitamin B_{12} deficiency in tapeworm carriers.

The Schilling test values were compared with the serum levels of vitamin B_{12}, 25 per cent of the tapeworm carriers with normal Schilling test values had pathological serum levels of vitamin B_{12}. This discrepancy does not seem to be purely one of methodology In these cases, the deficiency may thus be the consequence of some factor other than the impaired absorption only

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serum level of vitamin B_{12}, bone marrow findings, and neurological disturbance of the cases

[illegible] A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tapeworm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
1.6	< 3	10	98	17.9	typical	40	10	1	3	+
0.9		> 1,000	7	12.4	none	40	10	1	3	—
0.79	1.8	50	93	17 1	typical	45	15	6	8	+
		900	15	12.8	none					+
1 4	1 1	24	81	16.0	typical	40	25	3	4	+
0.07		125	6	12.6	none	40	25	2	4	+
0.37	1.5	120	83	16 4	typical	40	25	0	1	+
0	6	12	87	16.0	typical			6	5	+
0.15	3 8	< 10	98	17 1	typical	35	15	0	2	+
		38	22	13 1	none	35	15	0	2	+
0.37		> 1,000	3	12.2	none	35	15	0	2	—
< 0 3	0	60	92	15.8	typical					+
0.32	0	95	87	16.5	typical	35	15	5	6	+
0 03	6 6	60	58	14.8	typical	30	10	0	5	+
0 07	1.2	< 10	87	16.8	typical			1	8	+
0.15		> 1,000	15	12.5	none			1	8	+
0.34	0	< 10	91	18.5	typical	50	10	1	5	+
0.41	1.8	< 10	88	16 6	typical	65	45	0	3	+
0.28		300	32	13.7	none	65	45	0	3	—
0.01	0	12	93	15.4	typical	60	25	2	3	+
		600	19	13.0	none	60	25	0	2	+
0 5	4 4	10	98	18 0	typical	50	20	3	4	+
		700	13	12.7	none	30	20	0	4	—
0 08	0.1	< 10	83	16.2	typical	25	0	3	4	+
		> 1,000	10	12.5	none	25	0	3	4	+
0 03	0.3	170	77	15.8	typical					+
0.13	0.2	< 10	94	16.6	typical	50	10	2	6	+
0.92	0 7	12	94	17 7	typical	50	40	0	2	+
> 0.6	0	< 10	94	15 7	typical			0	6	+
0 05		15	22	13.3	none			5	7	+
0.42	2.8	150	81	16.3	typical					+
0 46	4 4	35	93	17.0	typical	50	15	3	4	+
0 1		90	5	12.5	none	50	15	1	5	—
0.52	3.0	< 10	94	16.9	typical	45	30	1	2	+
0.26		> 1,000	12	12 8	none					+

APPENDIX I

Age sex, month, peripheral blood count [illegible] A [illegible] test value, Schilling test value

Number of the case	Age	Sex	Month	Peripheral blood count							
				Haemoglobin	MCV	RBC	PCV	MCHC	MCH	Reticulocytes	Granulocytes
1	52	m	VI	2.9	0.9	8.3	92	35	32	2.2	2,500
			VIII	12.9	4.2	39	93	33	30	0 4	3,500
2	67	f	VII	3.0	0.59	9.0	153	33	51	1.0	1,300
			X	11.2	3.8	37.5	99	30	30	0.6	3 100
3	44	f	IV	3.5	1.04	11	106	32	33	0.5	900
			VI	13 1	4.35	39.5	91	35	30	0.5	3,000
4	45	f	VII	4.3	1 15	14	122	31	37	0.8	2,900
5	60	m	VI	4 4	1.25	15	120	29	35	0.5	3,000
6	60	f	VI	4 7	1.55	12	78	39	30	0.5	1,200
			VII	10.4	3.5	34	98	30	30	0.3	2,000
			VIII	11.9	3.8	38 4	100	31	31	0.3	2,400
7	84	m	VII	4.9	1.35	15	112	33	36	0.5	1,500
8	53	m	IX	5.2	1.55	14	91	37	33	2.6	4 400
9	62	f	VIII	5.4	1.85	18.6	100	29	29	4.2	2,500
10	62	m	VI	5.8	1.6	17	106	34	36	0	2,800
			VIII	10.8	3 67	36.7	100	29	29	0 6	7 100
11	69	m	VI	5.9	1.65	20.5	124	28	36	0	1 700
12	66	f	VI	5.9	1.8	19.5	108	30	33	6.8	1,000
			X	12.9	4 75	42	89	30	27		3,200
13	73	f	VI	6.1	1.9	18.7	98	33	32	0.7	3,000
			VIII	12.2	4 15	41.5	100	29	29	0.7	4,500
14	68	f	IV	6.1	1 75	20	107	30	35	0.9	2 400
			VI	10 1	2.85	34	119	29	35	0.6	4 400
15	76	m	VI	6.1	1.9	20	106	30	32	0 4	2,200
			X	13.3	4.05	43	106	31	33	0.9	3 400
16	69	m	VIII	6.1	1 9	20	106	30	32	0.4	8,400
17	64	m	V	6.3	1 7	21.5	127	29	37	1 4	1,300
18	25	f	III	6.9	1.85	22.5	122	30	37	2.3	2 600
19	58	f	III	6.9	1.86	21	114	33	37	1.6	4,500
			VII	12 75	4 7	42	90	30	27	0.7	3,600
20	59	f	VIII	7 1	2.1	22.5	107	31	34	0.6	2,800
21	38	m	V	7.3	1.9	22	116	33	38	0.2	1,500
			VII	15.2	5.35	48.5	91	31	28	0.6	5,200
22	75	f	VI	7 4	1.9	22	116	34	39	0.4	5,800
			VIII	11.2	3.95	38	96	29	28	0.7	4,600

Aser A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tape-worm eggs
						vibration		position		
			> 14 μ	mean		upper limb	lower limb	upper limb	lower limb	
0.14	8.9	160	76	15.3	typical	40	20	1	1	+
		650	11	12.9	none	40	20	1	1	—
0.12	0	160	91	16.5	typical					+
		> 1,000	15	12.8	none					—
0.13	0.8	< 10	93	17.5	typical	50	15	0	0	+
0.1		> 1,000	17	12.9	none	50	15	0	0	+
0.27	2.6	< 10	92	16.5	typical					+
		700	38	13.9	none					+
0.46	0.5	150	66	14.9	typical	50	10	2	2	+
0.04		650	13	12.8	none	50	10	2	2	+
0.22	0.6	10	92	16.7	typical	30	20	2	6	+
0.1		720	4	12.4	none	45	20	3	4	—
0.28	11.3	140	79	14.7	signs	50	10	0	2	+
0.2	1.3	240	77	14.9	typical					+
0.53	1.0	95	36	13.7	signs	33	10	1	3	+
0.2		> 1,000	21	13.1	none	33	10	1	3	—
0.26	12.6	15	59	14.1	signs	40	15	2	2	+
1.04	0.5	100	17	13.1	none	40	30	1	3	+
		> 1,000				35	35	1	3	+
0.62	4.4	250	18	13.0	none					+
2.1	18.1	120	10	12.4	none	45	20	0	4	+
		> 1,000	10	12.6	none	45	20	0	4	+
2.48	14.9	170	19	12.9	none	33	40	4	3	+
		> 1,000	10	12.6	none	33	40	2	1	—
0.49	8.2	85	14	12.8	none	45	30	0	2	+
1.4	4.6	43	78	16.0	signs	25	15	4	1	+
		> 1,000	11	12.5	none					—
> 0.6	25.5	80	24	13.1	none	50	30	0	3	+
		> 1,000	7	12.3	none	50	30	0	3	—
0.17	18.7	120	14	12.8	none	35	50	1	2	+
0.9	0.2	220	35	13.7	signs	45	20	2	4	+
0.12	0	60	81	15.0	none	40	20	0	3	+
0.15	0.5	60	22	13.0	none	35	0	0	2	+
0.22		> 1,000	9	12.8	none	35	0	0	2	—
1.6	10.0	135	9	12.6	none	60	40	0	3	+
0.4	7.0	240	69	15.3	signs					+
2.8		400	17	12.7	none					+
0.54	15.3	220	22	13.0	none	65	50	0	5	+
3.5		300	19	13.2	none	65	50	0	5	—

Number of the case	Age	Sex	Month	Peripheral blood							
				Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes
23	75	f	IV	7 4	2 15	24	112	31	34	3.8	4 100
			VII	12.2	4.3	41.5	97	29	28	0.5	4,500
24	67	m	V	7 7	2.2	24.5	112	31	35	0.5	4,600
			VII	12.6	4 1	43.5	106	29	30	0.8	2,300
25	46	m	VI	8.0	2.4	24	100	33	33	1.2	3,700
			VIII	12.6	4.3	40	94	31	29	0.8	3,200
26	64	f	VI	8.3	2.0	24	120	34	41	1 1	2,400
			VII	12 0	3.95	38.5	98	31	30	0 4	6,400
27	46	m	VI	8 7	3.02	32	105	27	29	0.8	4,900
			VIII	15.9	5.7	49.5	87	32	28	0.9	3,700
28	54	f	IV	8.9	2.9	31.5	109	28	30	0.7	3,000
			VI	13.3	4.3	42	98	31	31	0.4	1,600
29	76	f	VII	9 6	2.5	30	120	32	38	2 4	3,500
30	64	f	IV	9 7	3.5	37	106	26	28	0.9	3,000
31	66	m	VIII	10 1	3.2	33	104	30	32	0.8	7,000
			X	14.35	4 1	45	110	31	35		9,700
32	63	f	VII	10 1	2.95	32	108	32	34	0.5	2,500
33	41	f	V	10 1	3.2	35	110	28	31	1 7	6,700
			VII	11.0	3.6	35.5	99	31	31	2.6	4,300
34	51	m	VIII	10.1	3.5	36.5	104	28	29	1.0	6,300
35	48	f	IV	10.6	3.5	35	98	30	30	2 4	2,300
			VII	11 6	4 1	38.5	94	30	28	1 1	3,000
36	18	m	IV	10.6	4 15	40.8	98	26	26	1 6	5,200
			VII	10 6	4 15	40.8	98	26	26	1 0	10,800
37	40	f	II	10 6	3.5	37	106	28	30	2.1	11,300
38	72	f	VI	11 0	3.25	36	112	30	34	5.8	7,500
			VIII	12.9	4.2	40	96	32	31		4,300
39	47	f	III	11 0	3.3	35	106	31	33	1.5	2,000
			VI	11.35	4 0	37.5	94	30	28	1.8	4 400
40	36	f	VIII	11 0	3.65	36.5	100	30	30	1 7	8,100
41	68	m	VIII	11.2	3.7	39	106	29	30	1 4	6,300
42	47	m	VI	11.2	4 0	38	95	30	28	0.1	7,000
43	65	f	II	11.2	3.85	38	99	29	29	0.3	3,500
			VI	9 6	3.2	32	100	30	30	1 4	9,200
44	24	f	VI	11.2	3.95	40.5	102	28	28	0.5	3,800
45	17	f	VIII	11 35	3.7	38.5	104	29	31	1 7	5,600
			X	13.7	3.9	42	108	32	35	1.6	5,700
46	15	m	VIII	11.35	4 13	37	90	30	27	1.3	6,300
			X	12.0	4.35	40	92	30	28	1 1	3,500

[illegible] A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tapeworm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
1.1	6.7	800	25	13.6	none	45	10	2	3	+
0.27	0	28	28	13.4	none	20	0	1	2	+
0.21		> 1,000	5	12.5	none	20	0	1	2	+
1.3	0.3	140	15	13.1	none	50	0	0	3	+
		> 1,000	5	12.5	none	50	20	0	3	—
1.7	12.0	> 1,000	14	12.7	none					+
0.17	9.1	> 1,000	17	13.1	none	30	15	0	2	+
1.55	10.6	> 1,000	11	12.8	none	20	10	1	1	+
0.05	0	< 10	53	14.7	signs	40	0	0	4	+
0.4		190	13	12.8	none	50	0	3	3	—
0.7	23.3	200	11	12.8	none	50	20	0	6	+
2.0	8.0	110	20	13.3	none	70	50	0	2	+
		> 1,000	10	12.8	none	70	50	0	2	—
3.0	12.8	> 1,000	15	13.1	none	55	55	0	3	+
1.3	22.4	280	7	11.9	none	55	35	1	5	+
2.32	18.0	> 1,000	14	12.7	none	60	20	0	1	+
	8.0	100	16	12.8	none	30	10	0	3	+
0.78	13.4	110	12	12.4	none	65	25	0	2	+
0.36	4.0	52	11	12.8	none	20	40	0	1	+
0.4	0.7	300	28	13.6	none					+
1.42		550	9	12.4	none					+
2.2	16.2	650	28	13.5	none	50	35	2	2	+
		> 1,000	10	12.8	none	50	35	2	22	—
1.2	12.0	350	23	13.2	none					+
		400	19	13.1	none					+
0.91	13.0	11	35	13.8	signs	45	30	1	2	+
		> 1,000	16	12.9	none	45	30	1	2	+
2.0	4.4	> 1,000	30	13.4	none	55	30	0	0	+
		> 1,000	5	12.1	none	55	15	0	0	+
0.19	9.5	140	6	12.2	none	25	25	0	2	+
1.66	4.4	650	14	12.8	none	55	45	0	0	+
0.72	12.0	650	19	13.0	none					+
		> 1,000	14	12.8	none					—
3.3	19.6	500	30	13.6	none	50	30	0	2	+
0.62	11.3	70	24	13.3	none					+
		140	11	12.8	none					—
0.09	10.0	< 10	42	14.1	signs	50	20	0	0	+
0.17		140	27	13.8	none	40	20	0	0	+
0.4	0.7	150	32	13.8	none					+

Number of the case	Age	Sex	Month	P lph l bl d t							
				Haemo-globin	RBC	PCV	MCV	MCHC	MCH	Reticulo-cytes	Granulo-cytes
47	61	f	VII	11.6	4 05	38.5	95	30	29	1.6	6,100
48	74	m	V	11.6	3.75	39	105	29	31	0.9	8,600
			VII	9 6	3.35	33.5	100	28	29	1.5	9,900
49	57	f	III	11.6	3.75	35	94	33	31	1.0	4 400
			VI	10.6	3.6	36.2	102	29	29	2.3	9,000
50	57	m	VII	11.6	4 15	40.5	98	28	28	1 0	11,500
51	58	f	VII	11.6	4.25	40 6	96	28	27	1.2	6,900
52	55	f	V	11.6	3.95	38	97	30	29	0.6	4 400
53	57	m	II	11 7	3 45	37	108	32	34	1.0	6,500
			VI	14 75	5.25	48	92	30	28	0.3	6,400
54	64	m	V	11 7	3.2	37	116	32	37	0.3	4,900
55	16	m	V	11.9	4.0	38	95	31	30	1.3	6,000
			VII	11.2	4 1	39.5	96	28	27	3.2	6,200
56	54	f	VIII	11.9	4 15	39	94	30	29	2..	3,100
57	41	f	VII	11.9	4.5	42.5	94	28	26	1.5	4,200
58	19	m	V	11.9	4.3	40	94	32	28	0 7	2,600
59	58	f	VII	11.9	4.25	40	94	30	28	0.3	2,800
60	35	f	II	11.9	3.9	37	95	32	30	0.3	4 100
61	60	m	VI	12.0	4.55	43	94	28	26	0.8	6,500
62	75	f	VII	12.2	4.35	43	99	29	28	0 4	2,700
			X	14 75	4 7	48	102	30	31	0.6	2,900
63	18	m	VII	12.2	4 1	40.5	99	30	30	0.3	6,100
			X	12.9	4 1	37	90	34	31	1 4	4,000
64	75	f	VII	12.2	4.3	41.5	97	29	28	0 4	3,400
			X	12.2	4.25	43	102	28	29	1.5	4,800
65	60	f	IV	12.2	3.7	42	114	29	33	1 1	3 700
			VII	11.9	4 0	38.5	96	31	30	0.6	3,200
66	42	f	II	12.2	4 0	39	98	31	30	3 1	5,300
			VI	12.9	4.9	43	88	30	26	1.2	2,200
67	45	m	II	12.2	4 1	41	100	30	30	0.5	4,000
68	70	f	V	12.2	4.25	43	102	28	29	0.5	5,400
69	58	f	VI	12.35	4.55	44.5	98	28	27	0.9	4,200
			X	12.9	4.35	43	99	30	30	0.9	2,500
70	46	f	III	12.35	4.3	43.5	102	28	29	0 6	3,000
71	50	m	III	12.35	4.5	40	89	31	27	0.4	1,800
			VII	9.3	3.25	33.5	104	28	29	0.7	1,900
72	72	f	III	12.3	3 4	40	118	31	36	1.5	3,100
			VI	15.0	4.85	45.5	94	33	31	0.9	3,600
73	83	f	VII	12.35	4.35	43	99	29	28	0.7	2,900

Acer A	Schilling	Serum B_{12}	Macrocytes, diameter		Megaloblasts	Deep sensibility				Tapeworm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
1 1	6.7	800	25	13.6	none	45	10	2	3	+
0 27	0	28	28	13 4	none	20	0	1	2	+
0.21		> 1,000	5	12.3	none	20	0	1	2	+
1.3	0.3	140	15	13.1	none	50	0	0	3	+
		> 1,000	5	12.3	none	50	20	0	3	—
1.7	12.0	> 1,000	14	12.7	none					+
0.17	9.1	> 1,000	17	13.1	none	30	15	0	2	+
1.35	10.6	> 1,000	11	12 8	none	20	10	1	1	+
0 03	0	< 10	33	14 7	signs	40	0	0	4	+
0.4		190	13	12.8	none	50	0	3	3	—
0.7	23.3	200	11	12.8	none	50	20	0	6	+
2 0	8 0	110	20	13.3	none	70	30	0	2	+
		> 1,000	10	12.8	none	70	30	0	2	—
3.0	12.8	> 1,000	13	13.1	none	55	55	0	3	+
1.3	22.4	280	7	11.9	none	55	35	1	3	+
2.32	16.0	> 1,000	14	12.7	none	60	20	0	1	+
	8 0	100	16	12.8	none	30	10	0	3	+
0.78	13.4	110	12	12.4	none	65	25	0	2	+
0.36	4.0	52	11	12.8	none	20	40	0	1	+
0.4	0.7	300	28	13 6	none					+
1 42		350	9	12.4	none					+
2.2	16.2	650	28	13.5	none	50	35	2	2	+
		> 1,000	10	12.6	none	50	35	2	22	—
1 2	12 0	350	23	13.2	none					+
		400	19	13.1	none					+
0.91	13.0	11	33	13.8	signs	43	30	1	2	+
		> 1,000	16	12.9	none	43	30	1	2	+
2.0	4 4	> 1,000	30	13.4	none	55	50	0	0	+
		> 1,000	5	12.1	none	55	13	0	0	+
0 19	9.5	140	6	12.2	none	25	25	0	2	+
1.66	4 4	650	14	12.8	none	55	45	0	0	+
0.72	12.0	650	19	13.0	none					+
		> 1,000	14	12.8	none					—
3.5	19 6	500	30	13.6	none	50	30	0	2	+
0.82	11.3	70	24	13.3	none					+
		140	11	12.8	none					—
0.69	10.0	< 10	42	14.1	signs	50	20	0	0	+
0.17		140	27	13.8	none	40	20	0	0	+
0.4	0 7	150	32	13 8	none					+

Number of the case	Age	Sex	Month	[illegible]							
				Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes
47	61	f	VII	11.6	4 05	38.5	95	30	29	1.6	6,100
48	74	m	V	11.6	3.75	39	105	29	31	0.9	8,600
			VII	9 6	3.35	33.5	100	28	29	1.5	9,900
49	57	f	III	11.6	3 75	35	94	33	31	1 0	4 400
			VI	10.6	3 6	36.2	102	29	29	2.3	9,000
50	57	m	VII	11.6	4 15	40.5	98	28	28	1.0	11,300
51	58	f	VII	11 6	4.25	40.6	96	28	27	1.2	6,900
52	53	f	V	11.6	3.95	38	97	30	29	0 6	4 400
53	37	m	II	11 7	3 45	37	108	32	34	1.0	6,500
			VI	14 75	5.25	48	92	30	28	0.3	6,400
54	64	m	V	11 7	3.2	37	116	32	37	0.3	4,900
55	16	m	V	11.9	4 0	38	95	31	30	1.3	6,000
			VII	11.2	4 1	39.5	96	28	27	3.2	6,200
56	54	f	VIII	11.9	4 15	39	94	30	29	2.2	3 100
57	41	f	VII	11.9	4.5	42.5	94	28	26	1.5	4,200
58	19	m	V	11.9	4.3	40	94	32	28	0.7	2,600
59	58	f	VII	11.9	4.25	40	94	30	28	0.3	2,800
60	35	f	II	11.9	3.9	37	95	32	30	0.3	4 100
61	60	m	VI	12 0	4.5[illegible]	43	94	28	26	0.8	6,500
62	75	f	VII	12.2	4.35	43	99	29	28	0 4	2,700
			X	14 75	4 7	48	102	30	31	0.6	2,900
63	18	m	VII	12.2	4 1	40.5	99	30	30	0.3	6,100
			X	12.9	4 1	37	90	34	31	1 4	4 000
64	75	f	VII	12.2	4.3	41.5	97	29	28	0.4	3,400
			X	12.2	4.25	43	102	28	29	1.3	4,800
65	60	f	IX	12.2	3.7	42	114	29	33	1 1	3 700
			VII	11.9	4 0	38.5	96	31	30	0.6	3,200
66	42	f	II	12.2	4 0	39	98	31	30	3 1	3,300
			VI	12.9	4.9	43	88	30	[illegible]6	1.2	2,200
67	45	m	II	12.2	4 1	41	100	30	30	0.5	4,000
68	70	f	V	12.2	4.25	43	102	28	29	0.5	5,400
69	38	f	VI	12.35	4.55	44.5	98	28	27	0.9	4,200
			X	12.9	4.35	43	99	30	30	0.9	2,300
70	46	f	III	12.35	4.3	43.5	102	28	29	0 6	3,000
71	50	m	III	12.35	4.5	40	89	31	27	0.4	1,800
			VII	9.3	3.25	33.5	104	28	29	0 7	1,900
72	72	f	III	12.3	3 4	40	118	31	36	1.5	3 100
			VI	15.0	4.85	45.5	94	33	31	0.9	3,600
73	85	f	VII	12.35	4.35	43	99	29	28	0.7	2,900

Amr A	Schilling	Serum B_{12}	Metamyelocytes, diameter > 14 μ	Metamyelocytes, diameter mean	Megaloblasts	Deep sensibility, vibration: upper limb	Deep sensibility, vibration: lower limb	Deep sensibility, position: upper limb	Deep sensibility, position: lower limb	Tapeworm eggs
1.3	1.7	900	41	13.9	none					+
		> 1,000								+
2.8	4.3	12	42	13.9	none	45	20	0	2	+
		280	7	12.8	none	45	20	0	2	—
1.9	9.8	220	18	13.1	none	60	35	0	1	+
		> 1,000	10	12.6	none	60	35	0	1	—
1.7	7.8	> 1,000	32	13.2	none	0	0	0	3	+
		> 1,000	6	12.5	none	0	0	0	3	—
0.06	0.7	11	80	15.5	signs	50	25	2	6	+
		> 1,000	29	13.4	none	50	25	0	4	—
0.43	5.0	34	7	12.8	none	35	25	6	10	+
0.3	1.1	18	23	13.2	none	40	0	2	2	+
2.9	4.8	50	7	12.5	none	60	40	1	3	+
0.76	3.6	180	5	12.3	none	50	22	0	1	+
3.1	9.5	250	12	12.6	none	60	15	0	3	+
2.5	15.9	250	20	13.2	none	50	50	1	2	+
		> 1,000	11	12.8	none	50	50	1	2	—
2.7	18.2	1,000	19	12.7	none					+
0.82	7.4	180	26	13.2	none	55	33	0	1	+
> 0.8	3.0	160	38	13.8	none	50	20	0	0	+
2.7	6.2	250	28	13.6	none	50	25	0	3	+
0.37	2.4	350	37	14.8	signs	30	5	0	2	+
1.85	12.0	300	71	15.6	none	55	30	0	0	+
		900								
1.1	36.2	250	29	13.2	none	35	30	2	1	+
		350	9	12.5	none	50	35	2	3	—
2.3	8.0	11	67	15.1	none	30	20	1	3	+
		500	8	12.4	none	35	20	0	1	—
0.3	16.1	800	33	13.6	none	40	20	0	1	+
		130								+
0.1	0	22	10	12.8	none	55	30	0	3	+
0.35	27.2	38	8	11.8	none	35	20	2	5	+
1.8	2.8	250	12	12.7	none	45	20	0	0	+
0.82	11.4	300	39	13.9	none	30	15	0	3	+
		1,000	10	12.5	none	30	15	0	3	—
1.12	21.3	300	32	13.9	none	40	15	1	2	+
		> 1,000	6	12.3	none	40	15	1	2	—
0.53	13.1	84	47	14.1	none	45	15	1	5	+
0.3		170	15	12.8	none	45	15	5	5	—

Number of the case	Age	Sex	Month	P lph l bl d t							
				Haemo-globin	RBC	PCV	MCV	MCHC	MCH	Reticulo-cytes	Granulo-cytes
74	65	f	VIII	12 6	4.25	43	101	29	30	0.9	3,000
			X	14 1	4 1	45	110	31	34	1 1	4,200
75	59	m	VII	12.6	4.2	40	96	31	30		5,100
			X	12.0	3.82	41	108	29	31	1 7	6,000
76	33	f	VI	12.6	4.5	42	94	30	28	0.9	4 100
			VII	11.9	4 4	40	92	29	27	0.6	5,200
77	54	f	IV	12.6	4.33	40	92	31	29	1 7	4,600
			VI	14 1	4 75	43	90	33	30	0.9	4,200
78	77	f	IV	12.6	3.4	40	118	31	37	3.8	2,400
			VI	15.0	5.0	46.5	94	32	30	1 1	2 600
79	58	f	VII	12.6	4.25	41	97	31	30	0.5	2,700
80	67	m	II	12.6	4.3	44	103	28	29	3 1	6,400
81	20	f	VI	12.6	4.5	41	91	31	28	1.0	3 400
82	57	m	VI	12.6	4.55	45	99	28	28	1 1	4,600
83	46	m	IV	12 75	4.3	42	98	30	30	2 7	3,900
84	22	f	VII	12.9	4 45	40.8	92	31	29	0.5	4,800
			X	13 1	4 1	38.5	94	34	32	2.0	5,200
85	56	m	VIII	12.9	4.85	46	95	28	27	0 6	4,900
86	35	m	VII	12.9	4.8	42	88	30	27	1 4	3,700
87	70	f	VI	12.9	4.3	41	96	31	30	0.8	6,300
88	59	f	VI	12.9	4.2	41	98	31	31	0.9	3,700
89	74	f	VII	12.9	4 6	44	96	29	28	0.7	3,100
90	37	m	III	12.9	4 1	40	98	32	31	0.9	4 100
			VII	11.0	3.8	37.5	99	29	29	1.8	6,200
91	33	f	II	12.9	4.5	42	94	30	29	0 4	2,900
			VI	11.9	4 15	40.5	98	29	29	0.5	2,500
92	40	f	IV	12.9	4 18	42	100	31	31	0.8	3,100
			VII	13 7	5.9	47	80	29	23	0.8	3,400
93	35	f	IV	12.9	3.9	40	103	32	33	2.9	6,000
			VII	11.6	3.95	39	99	30	29	1.3	8,000
94	71	f	II	12.9	4 1	40	98	32	31	1.6	4,600
95	49	m	II	12.9	4.5	43	96	30	29	0.7	5,900
96	46	m	VI	12.9	4.8	45	94	28	27	1.0	6,300
97	40	m	VI	13 1	4 4	45	102	29	30	0.8	5,700
			VIII	11 7	4 15	38	92	30	28	0.2	9,300
98	42	f	III	13.1	4 6	42.5	93	31	28	0,3	2,600
			VI	11.9	4 4	43	98	28	27	1.2	5,100
99	62	m	IV	13.1	4.3	44.1	103	29	30	1.0	7 100
			VI	12.2	4 1	40	98	30	30	0.9	7,500

[illegible] A	Schilling	Serum B_{12}	Metamyelocytes no., diameter > 14 µ	Metamyelocytes no., diameter mean	Megalo-blasts	Deep sensibility: vibration, upper limb	Deep sensibility: vibration, lower limb	Deep sensibility: position, upper limb	Deep sensibility: position, lower limb	Tape-worm eggs
1.3	1.7	900	41	13.9	none					+
		> 1,000								+
2.8	4.3	12	42	13.9	none	45	20	0	2	+
		280	7	12.8	none	45	20	0	2	—
1.9	9.8	220	16	13.1	none	60	35	0	1	+
		> 1,000	10	12.6	none	60	35	0	1	—
1.7	7.6	> 1,000	32	13.2	none	0	0	0	3	+
		> 1,000	6	12.5	none	0	0	0	3	—
0.06	0.7	11	80	15.5	signs	50	25	2	6	+
		> 1,000	19	13.4	none	50	25	0	4	—
0.43	5.0	34	7	12.8	none	35	25	6	10	+
0.3	1.1	18	23	13.2	none	40	0	2	2	+
2.9	4.8	50	7	12.5	none	60	40	1	5	+
0.76	3.6	180	5	12.5	none	50	22	0	1	+
3.1	9.5	250	12	12.6	none	60	15	0	3	+
2.5	15.9	250	20	13.2	none	50	50	1	2	+
		> 1,000	11	12.8	none	50	50	1	2	—
2.7	18.2	1,000	19	12.7	none					+
0.82	7.4	130	26	13.2	none	55	33	0	1	+
> 0.6	3.0	160	30	13.8	none	50	20	0	0	+
2.7	6.2	250	26	13.6	none	50	25	0	3	+
0.37	2.4	350	57	14.6	signs	50	5	0	2	+
1.85	12.0	500	71	15.6	none	55	30	0	0	+
		900								
1.1	36.2	250	29	13.2	none	35	30	2	1	+
		350	9	12.5	none	30	35	2	3	—
2.5	8.0	11	67	15.1	none	30	20	1	5	+
		500	8	12.4	none	35	20	0	1	—
0.5	16.1	800	33	13.6	none	40	20	0	1	+
		150								+
0.1	0	22	10	12.8	none	55	30	0	3	+
0.55	27.2	38	8	11.8	none	55	20	2	5	+
1.8	2.8	250	12	12.7	none	45	20	0	0	+
0.82	11.4	300	39	13.9	none	30	15	0	3	+
		1,000	10	12.5	none	30	15	0	3	—
1.12	21.3	300	32	13.9	none	40	25	1	2	+
		> 1,000	6	12.3	none	40	15	1	2	—
0.55	15.1	84	47	14.1	none	45	15	1	5	+
0.5		170	15	12.8	none	45	15	3	5	—

Number of the case	Age	Sex	Month	P lph l bl d t							
				Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes
100	20	f	IV	13 1	4.2	42.5	102	30	31	0.4	3,700
			VI	11.2	4.2	37	88	30	27	0.9	5,900
101	62	m	VI	13.3	4.35	43.5	100	30	31	0.9	3,200
			X	14 1	4.55	43	95	32	31	1 0	5 700
102	55	f	VII	13.3	4.3	43.5	102	30	31	1 4	3,900
103	30	m	IV	13.3	4.9	45	92	29	27	1.0	4,900
			VII	13 7	5.05	46	92	30	27	0.6	2,900
104	32	f	VI	13.3	4.2	42	100	31	32	1 7	4,600
			VII	13.3	4.55	42.5	94	31	29	1..	3,800
105	50	m	VI	13.3	4.55	43	93	31	29	0.9	5,000
			VIII	11 9	4 0	35	88	34	30	1 7	3,800
106	35	m	IV	13.3	4.8	42	88	31	28	0.5	7 400
			VI	14 75	4.95	43	88	34	30	0 4	4,000
107	57	m	V	13.3	4 0	44	110	30	33	0.9	6,100
			VII	12.6	4 05	42	104	30	31	0.9	7 000
108	54	m	VI	13.3	4 1	40.5	99	33	32		6,100
109	77	f	VI	13.3	4 45	44	99	30	30	0 7	1,900
110	53	m	VII	13.3	4 45	43.5	98	30	30		5,900
111	59	m	VII	13.5	4..	40.8	97	33	32	1 0	2,800
112	56	f	V	13.5	4.25	42	99	32	32	2.4	7,000
			VI	12.9	4 4	43	98	30	29	1.5	7,500
113	46	m	III	13.5	4.25	42	99	32	32	0.8	4 100
			VI	13 7	4 75	46.5	98	29	29	0.9	4 600
114	62	f	V	13.5	4.2	42	100	32	32	0.5	3,900
115	72	f	VI	13 7	4.5	44	98	31	31	0.3	6,100
			X	13 7	4 4	43	98	32	31	1.2	5,400
116	63	f	VII	13 7	4.85	47.2	98	29	28	0.5	4 100
			X	13 1	4.25	43	102	30	31	0.6	7,300
117	46	m	V	13 7	4 75	44.5	94	31	29	1.5	2,700
118	66	f	V	13.7	4.36	42	97	32	31	0.8	2 700
			VII	12.6	4 05	40	99	31	31	1.5	3,400
119	26	m	IV	13 7	4 12	43	108	30	33	1.0	7 400
			VII	12.2	4 1	40.5	99	30	30	0.8	7 700
120	63	f	VI	13 7	4.95	49	99	28	28	2 4	6,200
			VII	14 1	5.25	50	96	28	27	0.7	5,800
121	60	m	III	13 7	4.5	43	96	32	30	1.8	3,100
			VI	12.6	4.3	42.5	99	29	29	0.9	8,600
122	63	f	VI	13 7	4.3	44	98	31	30	0.6	3,000
123	44	m	II	13 7	4 4	42	96	32	31	1.3	6,000
124	58	f	IV	13 7	4 0	41	103	33	34	3.3	4 600

Age A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tapeworm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
2.5	10.9	70	32	13.6	none	60	45	0	1	+
		> 1,000	4	12.6	none	60	45	0	1	—
0 07	17.2	165	33	13.6	none	50	15	0	4	+
0 28		> 1,000	10	12.7	none	50	15	0	4	—
0	17.3	36	19	13 0	none	30	15	0	2	+
2.6	17.0	> 1,000	31	13.3	none	70	40	0	3	+
		300				70	40	0	3	+
4 8	19.8	> 1,000	34	13.9	none	50	20	2	1	+
		> 1,000	18	12.5	none	40	20	2	1	—
0.3	6.2	180	15	12.9	none	55	35	0	4	+
		160	17	12.8	none	55	35	0	4	-
3 6	3 0	26	35	13 8	none	40	30	1	3	+
0.4		56	3	12.5	none	55	35	1	1	—
0.56	10.7	1,000	21	13.2	none	50	20	2	4	+
0 06		400				50	20	2	4	—
0 08	2.9	300	17	13.0	none	45	20	0	3	+
0.29	2.0	> 1,000		12.1	none	35	20	2	3	+
0 78	6.1	600	16	13.0	none	40	20	2	4	+
0.13	1.2	250	72	15 4	many	45	15	1	2	+
2.01	10 7	500	22	13.3	none	25	25	1	2	+
		> 1,000	9	12.3	none	35	25	1	2	—
0 8	8 2	125	50	14.2	many	50	30	0	1	+
		> 1,000	7	12 6	none	50	30	0	1	—
4 0	13.3	80	13	12.3	none	25	15	0	3	+
1 5	8 0	500	44	14.1	none	45	20	0	2	+
		> 1,000	7	12.4	none	45	20	0	2	+
3 5	5 3	350	41	14.0	none					+
		550	15	12.2	none					+
1.3	4 1	700	22	13.1	none	50	15	0	1	+
0 65	18.4	300	23	13.4	none	45	15	4	5	+
		> 1,000	8	12.2	none	45	15	8	5	—
0.76	1.0	< 10	45	14 1	many	65	35	1	2	+
		20	6	11 9	none	65	35	1	2	+
0.20	3.8	900	28	13.8	none	40	15	1	4	+
		> 1,000	8	12.5	none	40	15	1	4	—
0.40	8 6	[illegible]	39	13.8	none	40	13	0	4	+
		600				35	13	0	4	—
1.8	10.5	850	12	13 0	none	50	25	1	1	+
0 85	1 9	140	0	11.2	none	65	40	0	2	+
< 0.5	11.7	18	14	12.7	none					+
										+

Number of the case	Age	Sex	Month	P iph l bl d c t							
				Haemo-globin	RBC	PCV	MCV	MCHC	MCH	Reticulo-cytes	Granulo-cytes
125	51	f	IV	13 7	4 75	43	91	32	29	1.3	1,800
126	63	f	IV	13 7	4.8	46	96	30	29	1 1	2,900
127	53	f	VI	13 7	4 65	44.5	96	31	29	0.2	1,300
128	27	m	II	13.9	4.25	41	97	34	33	2.0	3,900
			VI	15.2	5.4	48.5	90	31	28	0.7	5,800
129	56	m	III	13.9	5.25	46	88	30	26	0 7	5,600
130	64	f	VII	14 1	4 75	46.5	98	30	30	0.5	4,000
			X	14.5	4.6	42	92	34	32	0.8	4,600
131	51	f	VII	14 1	5.2	47.5	92	29	27	0.8	1,800
132	69	f	IV	14 1	4 4	43	98	32	32	2 4	3,000
			VII	13.3	4 6	44.5	97	30	29	1.5	9,200
133	48	f	VI	14 1	4.85	47	97	30	29	2.2	14,000
			VII	14 1	5.2	49	94	29	27	1.6	3,400
134	65	f	VI	14 1	5.0	44.5	90	32	28	1 4	2 400
			VIII	12.2	4.25	37	88	33	29	1 1	3,000
135	35	f	IV	14 1	4.85	45	93	31	29	0.7	1,800
			VII	13.5	4 75	43	90	31	28	0.8	2,900
136	31	m	V	14 1	4 7	46	98	30	30	1 4	9,900
			VII	13 7	4.9	46	94	30	28	1.3	6,500
137	44	f	IV	14 1	5.0	47	95	30	28	0.8	6,500
			VI	13.3	4 4	43	99	31	30	0 4	5 400
138	55	m	IV	14 1	4 75	43	91	32	30	1.3	3,200
			VI	14.5	4.9	46	94	31	30	1 1	5,800
139	79	m	VII	14 1	4 75	46.5	98	30	30	0.6	2,500
140	42	m	VII	14.35	4.95	48.5	98	29	29	1.5	9 700
			X	15 0	4.9	46.5	96	32	31	0 4	9,200
141	48	m	VI	14.35	4.3	43	100	33	33	1 1	3,200
142	54	f	VI	14.35	4 75	46	97	31	30	1 4	3,600
143	71	f	VIII	14.5	5.3	48.2	92	30	27	1 1	2,300
144	59	m	III	14.5	4.3	47	110	31	34	0.8	5,200
			VI	14.5	5.2	48	92	30	28	1.0	5,200
145	56	m	VII	14.5	4.65	46.5	100	31	31	1.3	3,400
146	19	m	VII	14.5	5.35	48.5	91	30	27	0.7	3,600
147	34	m	VI	14.5	4.95	46.5	94	31	29	1.5	5,400
148	70	f	IV	14.5	5.0	46	93	31	29	1 1	4 100
149	50	m	VI	14.5	5.15	48.5	95	30	28	0.7	4,000
150	58	m	III	14 75	4.8	48	100	31	31	1.2	4,800
			VI	14 1	5 1	46	90	30	28	0.5	6,100

Acer A	Schilling	Serum B_{12}	Metamyelocytes, diameter > 14 μ	mean	Megaloblasts	Deep sensibility: vibration, upper limb	lower limb	position, upper limb	lower limb	Tapeworm eggs
>0.6	5.7	<10	12	12.7	none	40	20	3	4	+
2.58	14.3	16	11	12.8	none	30	20	1	1	+
2.2	8.8	140	8	12.5	none	40	20	0	5	+
0.82	1.3	16	40	14.1	signs	60	40	0	1	+
		35	6	12.4	none	60	40	0	1	+
0.42	0	>1,000	14	13.2	none	30	20	0	2	+
2.16	17.8	27	25	13.4	none	40	20	1	4	+
		700	10	12.9	none	40	20	1	4	—
0.12	2.4	<10	23	13.3	none	60	40	0	0	+
0.4	5.8	32	40	14.0	none	60	0	0	0	+
		160	18	12.4	none					—
0.35	1.4	18	24	13.4	none	40	25	0	2	+
		70	5	12.1	none	40	25	0	2	+
1.76	15.6	220	17	13.3	none	40	10	2	4	+
		300	12	12.7	none	40	10	2	4	—
2.1	18.6	22	20	13.1	none	45	30	1	6	+
		>1,000	11	12.5	none	45	30	1	6	—
3.63	6.5	>1,000	20	12.7	none	65	30	0	2	+
						65	30	0	2	+
0.8	0.6	>1,000	16	12.9	none	55	25	0	2	+
		600	4	12.1	none	35	30	0	2	—
3.9	12.0	20	41	14.1	none	40	20	2	8	+
		320	17	13.1	none	15	15	3	5	+
>0.6	1.3	>1,000	39	13.8	none					+
0.77	15.8	600	27	13.4	none	60	30	0	3	+
		630								+
1.3	8.0	700	16	13.0	none	35	10	4	4	+
1.7	5.0	220	15	13.0	none	40	10	0	3	+
0.22	16.3	>1,000	23	13.4	none	25	10	0	4	+
0.6	11.0	120	46	14.2	none	40	20	1	2	+
		900	8	12.4	none	40	20	1	2	+
1.8	15.9	650	23	13.4	none	45	20	2	1	+
0.13	15.1	350	23	13.2	none	45	35	2	3	+
1.1	7.7	330	16	13.1	none	40	20	0	1	+
0.4	23.0	>1,000	13	12.5	none	60	8	3	5	+
1.1	18.2	800	14	12.9	none	45	30	0	4	+
0.37	0.3	11	22	13.4	none	35	25	3	4	+
0.44		1,000	6	12.8	none	30	25	2	3	—

Number of the case	Age	Sex	Month	P lph l bl d t							
				Haemoglobin	RBC	PCV	MCV	MCHC	MCH	Reticulocytes	Granulocytes
151	64	m	VI	14 75	5 1	48.5	96	31	29	1.0	2,600
			X	12.45	4 4	41.5	93	30	29	0.5	2,800
152	51	m	III	14 75	4 6	45	98	33	32	1.0	5,000
153	35	m	V	15 0	5.2	48	93	31	29	0.9	4,500
154	42	m	IV	15.0	5.25	48	92	31	29	1.5	7,900
155	21	m	III	15.0	5.1	47	93	32	29	0.6	4,300
156	69	m	VI	15 0	5.5	49.5	90	30	27	0 6	7 700
157	53	f	VI	15.0	5.55	50	90	30	27	0.6	2 100
158	62	m	VIII	15.0	5.5	49	89	30	27	1.5	3 400
159	23	m	III	15.0	5 1	50	98	30	29	0.4	2 100
			VI	15.0	5.4	50	92	30	28	0.2	4 700
160	52	f	IV	15.0	4 75	45	95	33	32	1 6	2,800
			VII	13.3	4 7	44	94	30	28	1.2	3,000
161	50	m	V	15.0	4.95	45	92	33	30	1.9	4,000
162	66	f	VII	15 0	5.05	46.5	92	32	30	1.0	5,900
			X	12.9	4 4	42.5	97	30	29	1.0	6,000
163	52	f	VII	15.0	5.3	49.5	94	30	28	1.2	6,100
			X	15.0	4.9	49	100	30	31	1.9	7,000
164	50	m	VII	15.2	4.95	45	91	33	31	0.5	2,000
165	63	m	IV	15.2	5 1	49	97	31	30	0 7	4,000
166	44	m	V	15.2	4.9	46.5	96	32	31	0.8	4,200
167	56	f	VII	15.2	5.55	50	90	30	27	0.2	3 600
			X	13.5	4.80	45	94	30	28	1 1	7,000
168	37	m	IV	15 4	5.55	48	87	32	28	1.2	4,500
			VII	15 4	5.35	50.5	94	30	29	1.6	3 400
169	68	f	V	15 4	5 1	49	97	31	30	0.9	3,800
			VII	15 4	5 4	48	89	32	28	0.4	4,200
170	29	m	II	15 4	5.5	48	88	32	28	1.9	6,600
171	17	m	II	15.4	5.2	47.5	92	32	30	0.6	3,700
172	45	m	II	15.4	4.95	46	93	33	31	1.9	5,400
173	56	m	VII	15.9	5.7	53	93	29	28	0.4	4,800
			X	15 4	5.2	51	98	30	30	1.2	5 400
174	23	m	III	15.9	5.35	50	94	32	30	0 4	4,300
			VII	14 75	5.2	47	91	31	28	0.9	3,000
175	50	m	VIII	15.9	5 6	54.5	98	29	28	1.5	8,500
176	52	m	VI	15.9	5.5	51	96	31	30	0 7	2,800
177	27	m	IV	15.9	5 4	49	91	32	29	0.4	3,000
178	16	f	III	15.9	5 45	52	96	30	29	0.8	4 000

Aner A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tapeworm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
0.33	10.8	1,000	27	15.5	none					+
		600	11	12.7	none					—
0.91	15.2	18	4	12.1	none	55	30	0	2	+
0.6	4.0	650	6	12.5	none					+
0.54	5.1	100	14	12.5	none					+
2.3	16.1	400	34	13.5	none	55	50	0	0	+
0.38	15.7	> 1,000	16	12.0	none	45	10	0	6	+
1.2	16.4	650	10	12.6	none					+
0.02	10.2	> 1,000	16	13.0	none	60	30	0	3	+
1.08	0	220	25	13.2	none	50	25	0	4	+
		600	5	12.2	none	50	25	0	2	+
> 0.6	3.2	350	29	13.5	none	50	35	0	4	+
		> 1,000								—
2.6	9.6	300	29	13.5	none	45	10	1	6	+
1.9	10.8	500	25	13.4	none	55	15	2	2	+
		500	9	12.8	none	55	15	2	2	—
1.8	12.7	450	34	13.5	none	55	30	1	3	+
		900	5	12.4	none	55	50	1	3	+
1.76	16.7	1,000	14	13.0	none	50	20	0	2	+
2.79	2.5	> 1,000	14	13.1	none	50	25	0	0	+
2.45	3.4	> 1,000	12	13.0	none	50	35	3	4	+
1.1	2.4	140	28	13.5	none	55	40	2	3	+
		> 1,000	19	13.2	none	55	40	2	3	+
2.9	16.2	> 1,000	32	13.8	none	60	40	2	2	+
		> 1,000	10	12.6	none	60	40	1	2	—
0.21	2.9	32	20	13.4	none	50	30	1	4	+
0.06		16	9	12.9	none	50	50	1	5	—
1.0	6.3	135	8	12.1	none	40	40	0	0	+
1.78	14.8	260	6	11.4	none	50	35	0	3	+
0.24	7.1	115	11	12.1	none	60	40	0	0	+
2.12	17.9	120	28	13.4	none	33	25	1	3	+
		200	7	12.3	none					—
3.3	17.7	70	53	14.4	none	50	20	0	0	+
		> 1,000	10	12.6	none					—
0.18	2.7	> 1,000	5	12.3	none	60	30	0	0	+
0.72	3.2	85	18	13.1	none	40	15	0	2	+
0.4	2.2	100	9	12.6	none	55	43	3	2	+
3.5	4.1	> 1,000	16	12.5	none	50	42	0	6	+

Number of the case	Age	Sex	Month	P lpk l bl d t							
				Haemo-globin	RBC	PCV	MCV	MCHC	MCH	Reticulo-cytes	Granulo-cytes
179	58	m	IV	16.2	5 45	50	91	32	30	1 1	2,300
			VII	15.9	5.3	50	94	32	30	1.5	3,600
180	30	m	VII	16.5	5.5	51	92	32	30	1 1	5,500
181	31	m	II	16.5	5.35	51.5	96	32	31	0.7	3,100
			VI	17.3	5.9	54	92	32	29	1 7	4,500
182	54	m	VI	16.5	5.2	55	106	30	32	2.0	5,500
183	23	m	V	16 7	5 7	52	92	32	29	1 0	4 700
184	63	m	VI	17.0	5 7	55	96	31	30	0.6	3,900

Assay A	Schilling	Serum B_{12}	Metamyelocytes, diameter		Megaloblasts	Deep sensibility				Tape-worm eggs
						vibration		position		
			> 14	mean		upper limb	lower limb	upper limb	lower limb	
1.2	17.0	> 1,000	25	13.5	none	45	25	1	1	+
		> 1,000	11	12.6	none	45	25	1	1	+
3.6	10.0	18	27	13.4	none	45	30	1	4	+
0.75	21.5	280	16	12.7	none	80	45	0	0	+
		> 1,000				60	45	0	0	+
0.56	5.2	70	8	12.6	none	50	40	0	4	+
0.98	12.2	350	8	12.4	none	65	40	2	2	+
0.38	5.4	150	9	12.7	none	50	20	1	4	+

APPENDIX II

PRINCIPAL DISEASES OF TAPEWORM CARRIERS

The list has been made according to the records of the attending physicians.

Infectious diseases

Fish tapeworm infection solely cases 29, 32 35 45 59 61 62 69, 70 73 74, 76 78 79, 81 84, 87 89 98 101 106, 109, 111 114, 116 118 133 135 142 157 163 174, 181

Other infections cases 100 152 155 180

Neoplastic diseases cases 31 34, 48 54, 88 90 99 112 129, 172

Allergic diseases cases 94, 120.

Endocrine diseases cases 64, 92 97 103 107 113 125, 126 127 131 134, 143 167 169, 178

Diseases of the nervous system cases 80, 147 153 154, 177

Diseases of the circulatory system cases 32 37 38 46 49, 55 71 80, 105 119, 124, 130 138, 140 141 145, 149, 156, 158, 159, 160, 161 173 176, 182 183.

Diseases of the respiratory system cases 36 40 42 52 58, 66 67 72 75, 82 83, 95, 121 123 136 139 146, 162 164, 165, 175 184.

Diseases of the digestive system cases 50, 51 56 65, 68 86 96, 110 128 132 144 166 168.

Diseases of the kidney and urinary tract cases 39, 43 44, 47 57 60, 63 93, 102 108, 115 170, 171

Diseases of the skin cases 41 53 85, 104, 137

Diseases of the joints cases 77 122 148, 151

Miscellaneous symptoms cases 91 117 150 179.

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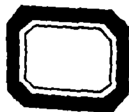
SUPPLEMENTUM 373

Balance Studies on Protein Metabolism in Normal and Uraemic Men

Effect of diet, bed rest and anabolic steroids

BY

F. H. WOLTHUIS M.D



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BALANCE STUDIES ON PROTEIN METABOLISM IN NORMAL AND URAEMIC MEN

From the Vereniging voor Ziekenverpleging, Prinsengracht, Amsterdam
and
the University Medical Clinic (Head: Prof. Dr. J. G. G. Borst), Binnengasthuis, Amsterdam

BALANCE STUDIES ON PROTEIN METABOLISM IN NORMAL AND URAEMIC MEN

EFFECT OF DIET, BED REST AND ANABOLIC STEROIDS

by

F. H. WOLTHUIS, M.D.

1961
AMSTERDAM

1961
SCHELTEMA & HOLKEMA N.V
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INTRODUCTION

The treatment of uraemic patients with calorically adequate diets containing a minimal quantity of proteins has for its purpose the utmost restriction of the formation of protein catabolites that have to be excreted by the kidneys.

In cases of chronic uraemia the diet must also fulfil a second condition which is contradictory to the first: it must contain enough proteins to ensure that the nitrogen balance does not permanently remain negative.

It is therefore necessary that a compromise be found, in such a way that a minimal quantity of protein catabolites is formed while the nitrogen balance just retains its equilibrium.

The purpose of the present investigation was to explore the factors that influence protein catabolism during the administration of no-protein and low-protein diets. For purposes of comparison, a study was also made of the effect of some of these factors when diets with a normal protein content were given.

The following aspects were studied:

(1) The influence of the length of the period during which the low-protein diet was administered. This includes the effect of protein depletion.

(2) The influence of the caloric value of the diet.

(3) The effect of bed rest.

(4) The significance of carbohydrates and fats as sources of calories, and the specific influence of carbohydrates and fats.

(5) The influence of the simultaneous or non-simultaneous intake of proteins and carbohydrates or fats.

(6) The effect of the food in a few large meals, as compared with the effect of many small meals spread over the day, the total quantity of food being equal.

(7) The influence of the quantity of NaCl in the food.

(8) The effect of anabolic steroids.

This investigation was carried out in 13 healthy persons and 1 patient with chronic nephritis. The diets were strictly standardized and usually were analysed. The total excretion of nitrogen, sulphur, phosphorus and potassium was determined in the urine and the faeces. Furthermore, the renal excretion of urea, ammonia and creatinine was measured.

we call this quantity x, the formula is

$$x = \frac{100k - g}{k - r}$$

in which k, g and r represent the ash contents of faeces with bone meal mixed faeces and faeces without bone meal, respectively. In view of the small differences in the quantities of faecal nitrogen that are found this method would appear to be somewhat over-elaborate even for accurate balance tests.

(2) The dyes have a disturbing influence on certain chemical determinations. The colorimetric determinations of sulphur and phosphorus that had been selected could not be used when carmine red or animal carbon had been added to the food.

Because of these objections, an attempt was made to mark the faeces by adding two coloured glass beads to the first meal of a given period. It was found, however that these beads did not always reappear with the same defaecation. In one experiment, the subject took two red beads first, and two green beads 15 days later. A low-residue diet was taken. The first red bead appeared 3 days after the beginning of the experiment, with the first defaecation but the second red bead appeared together with the first green bead with the fourth defaecation, on the third day after swallowing the green beads. Where this red bead hid itself for 18 days remains a mystery.

We have not succeeded in finding an accurate method for marking faeces that did not disturb the determinations. As a rule a low-residue diet was given, so that homogeneous faeces were obtained that differed considerably from the faeces before and after the experiment. The accuracy could be enhanced by instructing the subject to refrain from food for 12 hours before and after the experiment even then it often proved impossible to say to what period a given quantity of faeces belonged, so that it was necessary to collect the whole of the faecal excretion over several periods or over the duration of the whole experiment. In general this was of little importance, because the faecal excretion represented only a small part of the total excretion but where phosphorus and calcium were concerned it was often a considerable disadvantage, because in the case of these elements a considerable quantity may be excreted with the faeces.

The output of urine also is of influence in the study of nitrogen balances. This influence is greatest when little urine is produced, decreases rapidly with increasing output and has practically ceased when more than 2 ml. per minute are voided (PETERS and VAN SLYKE, 1946).

PETERS and VAN SLYKE (1946) have pointed out that in assessing nitrogen balances the alterations of the composition of the blood must be taken into account. This is especially true in the case of substances that diffuse readily such as urea. Approximately 80 % of blood consists of water and the water content of the body is approximately 60 %, so that the difference of the quantity of urea per kg. body weight is about 60/80 × 100 = 75 % of the difference of the quantity of urea per litre of blood (PETERS and VAN SLYKE, 1946; BLAND 1957).

The influence of a diet on protein metabolism often becomes evident only after it has been administered *over a period of considerable length*. The importance of this time factor has been demonstrated by LAGIER and DEMOLE (1953), who kept

B

DATA FROM THE LITERATURE

I. Nitrogen balance marking of faeces

A nitrogen balance based on intake with the food and excretion in the urine and faeces should be slightly positive because perspiration nasal secretion exfoliated skin hairs and nails are not taken into account. Under normal conditions, approximately 0.3 g. nitrogen per 24 hr are contained in the sweat of an adult individual living in a moderate climate in a hot climate and with increased perspiration this may rise to 0 9 g (BOST and BORGSTROM 1926–1927). In a steam bath for 2 to 4 hr 0 5–0 8 g. nitrogen may be secreted with the sweat (PETERS and VAN SLYKE, 1946 MAJOOR 1953)

In several species of animals it has been demonstrated that on a protein free diet, the faecal nitrogen consists of a constant fraction probably resulting from intestinal secretion, and a variable fraction which depends on the dry weight of the ingested food (PETERS and VAN SLYKE, 1946) Human faeces contain nitrogen in direct proportion to the amount of indigestible food that is taken (MENDEL and FINE, 1912 HINDHEDE 1913 MITCHELL, 1926), but there is no correlation between the quantity of nitrogen in the food and in the faeces.

The custom of earlier investigators, who used to estimate the faecal nitrogen as one-tenth of the nitrogen in the food is therefore incorrect. For practical purposes, a mean faecal excretion of 1.3 g. nitrogen per 24 hr may be accepted as a reasonable estimate

In patients with an increased urea concentration of the blood the faecal excretion of nitrogen is hardly increased unless diarrhoea occurs (MOSENTHAL, 1915 PETERS and VAN SLYKE, 1946), but even in the latter case the quantity of nitrogen in the faeces is of little significance MAJOOR (1953) points out that when the blood urea concentration is 2 g. per litre, only 1 g. urea is excreted with 500 ml. of faecal fluid.

For accurate balance tests, analysis of the faeces is necessary However the first difficulty encountered in that case is how to mark the faeces satisfactorily There are two objections against staining with carmine red or animal carbon

(1) When these dyes are administered orally we find that portions of the food ingested both before and after the period in question are also coloured so that a sharp distinction becomes impossible VOIT and KORKUNOFF (1895) have solved this difficulty by adding bone meal to the food during the experimental period, using a complicated formula to determine how much faeces without bone meal were present in a quantity of mixed faeces. If

we call this quantity x, the formula is

$$x = \frac{100k - g}{k - r}$$

in which k g and r represent the ash contents of faeces with bone meal, mixed faeces and faeces without bone meal re spectively In view of the small differences in the quantities of faecal nitrogen that are found this method would appear to be somewhat over-elaborate, even for accurate balance tests.

(2) The dyes have a disturbing influence on certain chemical determinations. The colorimetric determinations of sulphur and phosphorus that had been selected could not be used when carmine red or animal carbon had been added to the food.

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excretion is partially dependent on the quantity of nucleoproteins in the food, but does not vary with alterations in the total protein metabolism.

(5) *Creatinine*

The principal investigations concerning the production of creatine were carried out by BLOCH and SCHOENHEIMER (1940, 1940) and by BORSOOK and DUBNOFF (1940, 1940, 1941). Renal tissue is capable of synthesizing guanido-acetic acid from arginine and glycine, while in the liver this di-peptide can be methylated to creatine. VIGNEAUD, COHN, CHANDLER, SCHECK and SIMMONDS (1941) demonstrated that in the liver methionine supplies the required methyl group.

BLOCH and SCHOENHEIMER (1939) in rats recovered the heavy nitrogen of orally administered creatine exclusively in the form of creatine and creatinine. Moreover the proportion between labelled and normal creatine in the urine was the same as that which could be demonstrated for the creatine in the muscles, leading to the conclusion that creatinine originated from tissue creatine.

The quantity of creatinine in the urine depends mainly on the total mass of muscle tissue and is therefore constant. For this reason the creatinine excretion is often used as an extra check on the accuracy of the urine collection.

(6) After the nitrogen from the 5 above mentioned substances in the urine has been determined it is found that the total ... till a little less than the total quantity of nitrogen. Little is as yet known concerning the nature of the substances that cause this difference or about their origin.

III. Protein metabolism

(1) *Protein depot and protein depletion*

Under normal conditions, when an adequate quantity of protein is consumed the quantity of nitrogen excreted is exactly the same as the quantity ingested. When more protein is administered, the balance is temporarily disturbed. Over a brief period, additional nitrogen is retained, the quantity of which decreases day by day until the equilibrium is again achieved. Part of the retained nitrogen forms tissue protein, a smaller part is left in the body in the metabolite form. The enhanced excretion can be achieved only through a higher blood concentration of these metabolites (urea) (PETERS and VAN SLYKE, 1946).

From these observations, two conclusions may be drawn

1) the body is capable of forming a protein depot

2) the depot formed is limited.

Under normal conditions, therefore maintenance of a positive nitrogen balance in the adult subject is not possible. During the growth period and in persons who previously have been underfed or have lived on an extremely low-protein diet, it is possible to retain nitrogen for a long time. PETERS and VAN SLYKE (1946) in this connection state "There is a certain similarity between the nitrogen metabolism during recovery from malnutrition and during growth. In both, the tendencies to retain nitrogen and to synthesize tissues are more active than they are in the normal adult. Patients who lose large quantities of protein with the urine also respond as underfed subjects. When they are given a calorically adequate diet and sufficient

healthy persons on diets in which the quantity of protein differed to a varying extent from what these persons took previously The smaller the difference in alimentary protein the sooner equilibrium of the nitrogen balance was achieved.

II. Protein metabolites and total nitrogen in the urine

In the urine the following nitrogen-containing components are present

(1) *Urea*

The nitrogen from urea represents the main part of the nitrogen excreted through the kidneys. The quantity of urea that is secreted per day depends on the composition of the food and on the rate of protein decomposition in the body Urea diffuses readily through the cellular membranes the concentration in the cells and the plasma is the same (PETERS and VAN SLYKE, 1946)

When amino-acids are added to liver tissue in vitro urea is produced (KREBS, 1933) KREBS and HENSELEIT (1932) demonstrated that urea can also be synthesized from carbonic acid and ammonia with the aid of slices of liver tissue, whereas with other tissues the synthesis does not occur By addition of ornithine which acts as a catalyser the production of urea can be greatly enhanced Using isotopes it was possible to confirm the ornithine cycle of Krebs and Henseleit (FOSTER, SCHOENHEIMER and RITTENBERG 1939), and a relationship has been demonstrated between the urea production and the tri carboxylic acid cycle in which catabolites of protein carbohydrate and fat come together (RATNER, 1949 RATNER and PAPPAS, 1949 RATNER and PETRACK 1951)

(2) *Ammonia*

This substance is formed by deamination of amino-acids. The main part is used for urea production The excretion in the urine in the form of ammonia salts, increases in proportion to the organisms need of bases Consequently in cases of acidosis, the amount of ammonia in the urine is almost always increased (PETERS and VAN SLYKE, 1946) The chemical transformations connected with this phenomenon take place in the kidney (BENEDICT and NASH 1926)

If the quantity of ammonia increases, the urea secretion is diminished which does not mean that ammonia is formed from urea. The sum of the two remains the same (PETERS and VAN SLYKE, 1946)

(3) *Amino-acids*

Free amino-acids and small peptide complexes are found in the urine of normal persons as well. The quantitative proportions of the amino-acids in the urine are different from those in the blood. Also there are important individual differences. The total alpha-amino nitrogen excretion amounts to approximately 0.5 g. per day viz. from 1 1 g. free amino-acids and from 2 g. peptide complexes, such as glutathion (WHITE, 195?) The quantity of amino-acids excreted depends on the food but in practice this influence is of little importance. In cases of severe hepatic affection, the excretion of amino-acids may be increased

(4) *Uric acid*

This substance is formed as the end product of the purine metabolism. The

excretion is partially dependent on the quantity of nucleoproteins in the food, but does not vary with alterations in the total protein metabolism.

(5) *Creatinine*

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proteins to cover the usual metabolism plus the *quantity that is lost with the urine* the daily protein catabolism can be greatly reduced

When the nitrogen balance is normal and less protein is administered the balance becomes temporarily negative. *Some time afterwards*, equilibrium is re gained at a lower level

LIEBIG s theory concerning the protein metabolism was rejected by VOIT (1869) on the basis of experiments in dogs which after withholding of food initially excreted a large quantity of nitrogen which showed a considerable decrease in a few days time This is explained by assuming that in addition to the tissue protein there exists a circulating protein which is labile, is quickly disintegrated during fasting and is not used for the building up of tissues. It is only after this protein has been used up that the tissue protein which is much more stable, is disintegrated at a much *slower rate.*

FOLIN (1905) has elaborated methods *for the determination of the various types* of nitrogen in the excreta. This inspired a theory which for many years has domi *nated the views concerning protein me* tabolism Folin observed that certain substances in the urine (urea anorganic *sulphur compounds) were to a consider* able extent dependant upon the diet whereas other *substances were not de pendant upon what was ingested with the* food and were always excreted in constant quantities by a given test subject (cre *atinine, organic sulphur)* He advanced the opinion therefore that a precise distinction must be made between two types *of protein metabolism*, viz. an endogenic and an exogenic type. The quantities of creatinine and organic sulphur constitute a measure of the endogenic *metabolism* the rate of which depends on wear and repair of the body protein The term exogenic metabolism on *the other hand*, is used by Folin for the consumption of protein from the diet According to this theory there is a *distinct difference be* tween the labile exogenic metabolism and the static endogenic metabolism of *protein It is only a small constant part* of the body proteins which, due to wear has to be supplemented from the diet, while no other changes occur in the tissues The remaining protein in the diet is catabolized

As we shall argue in due course, FOLIN s theory can no longer be accepted in its entirety However his obs rvations of the essential differences between the nitrogen-containing components in the urine have been of fundamental importance. Further more they confirmed observations made by VOIT (1869) in fasting dogs During the *early phase* of a period of a low-protein diet a rapid daily decrease of the urea production is seen while afterwards the *protein* catabolism decreases at a much slower rate. Apparently therefore when there is a normal nitrogen balance the body *contains* a quantity of protein which can be rapidly catabolized The significance of *this depot protein* appears clearly from the experiments of CUTHBERTSON McGIRR and ROBERTSON (1939), who observed that the nitrogen *excretion in rats in* creased considerably after a bone had been fractured This nitrogen loss is con *siderably greater than* might be expected from local destruction of tissues and

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(2) *Dynamic equilibrium, the difference in half life time between different body proteins*

Experiments with protein metabolites labelled with isotopes have shown that FOLIN's hypothesis cannot be wholly maintained. In this field, work of prime importance has been done by SCHOENHEIMER school. Experiments have been carried out with orally administered amino-acids which had been labelled in different ways (N^{15}, C^{14}, S^{35}, deuterium). The first finding was that catabolites of less than 50 percent of the labelled amino-acids were excreted with the urine (SCHOENHEIMER, RATNER and RITTENBERG, 1939), while the body weight remained unchanged and the nitrogen balance was maintained. In other words, that part of the isotopes had been replaced by normal nitrogen from the food or from the body.

Approximately one third of the N^{15} incorporated into the tissues proved still to be linked to the same amino-acid, whereas the remainder had been incorporated in other amino-acids. A small part of the heavy nitrogen was linked to amino-acids circulating in the body. It was, however not linked exclusively to the amino-acids with which the N^{15} had been administered to the laboratory animals.

When labelled ammonium salts were administered, the isotopes were recovered in all amino-acids with the exception of lysine (SCHOENHEIMER and RATNER, 1939; FOSTER, SCHOENHEIMER and RITTENBERG, 1939; RITTENBERG, SCHOENHEIMER and KESTON, 1939; RATNER, WEISSMAN and SCHOENHEIMER, 1943). The two dicarboxylic acids, in particular (glutamic acid and asparaginic acid), presented an intense activity in incorporating heavy nitrogen.

SCHOENHEIMER, RATNER and RITTENBERG (1939) administered doubly labelled L-leucine to rats: N^{15} for the amino-group and deuterium for the CH skeleton. From the body protein an L-leucine was isolated that showed an N^{15}-deuterium proportion which differed from that of the amino-acid from the food, in favour of the deuterium. This is possible only when part of the N^{15} has been replaced by non-labelled nitrogen. The non-biological D-leucine was administered in the same way and in these cases heavy nitrogen was also transmitted to other amino-acids, but to a much smaller degree than had been observed with L-leucine.

TARVER and SCHMIDT (1942) were able to demonstrate resynthesis of protein in fasting animals. Methionine labelled with S^{35} was administered to dogs and rats which had fasted for 8 days. Subsequently the isotope was demonstrable in the proteins of many organs.

The animal experiments could be confirmed by experiments with tissue cultures and even with tissue homogenates (SHEMIN, LONDON and RITTENBERG, 1950).

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N^{15} content of protein nitrogen obtained from different organs after feeding L-leucine and glycine (25 mg N per day)
(Calculated for 100 atom per cent N^{15} in compound administered)

organ	after feeding L leucine	after feeding glycine
serum	1 67	1 78
haemoglobin	0.29	0.46
liver	0 94	1 40
intestinal wall	1 49	0 98
kidney	1.38	—
heart	0.89	—
spleen	1 10	—
testes	0.77	—
skin	0 18	—
muscle	0.31	0.29

BORSOOK DEASY HAAGEN SMIT KEIGHLEY and LOWY (1950) treated mice intra venously with glycine, leucine or lysine labelled with C^{14} These amino-acids disappeared from the blood within ten minutes, while within half an hour 75 / could be demonstrated in the proteins of the intestines. Expiration of labelled CO also started soon Soon *after* the appearance of the labelled amino-acids in the abdominal organs they could also be demonstrated in the serum proteins.

GREENBERG and WINNICK (1948) carried out an investigation in rats which were treated intravenously with 25 mg. labelled glycine in order to determine the per centage of C^{14} per gramme of organic protein after fifteen minutes and after six hours, respectively They found for the intestinal wall 0 3 and 3 7 for the bone marrow 0.2 and 2.15 for the liver 0.25 and 2.05 for the kidneys, 0 15 and 1 95 for the plasma, 0 05 and 1 8 for the spleen 0 05 and 1 45 for the lung, 0 15 and 1.25 for the testes, 0 05 and 0.5 for the muscles 0 0 and 0 15 for the erythrocytes, 0 0 and 0 15 and for the brain, 0 0 and 0 1

Labelled amino-acids incorporated in protein are replaced again by non-labelled substances. From the rate at which this happens, SPRINSON and RITTENBERG (1949) have calculated the half life time of proteins. The rat replaces half the total body protein in 17 days, the proteins of plasma and visceral organs in 6 or 7 days and those of the carcass in 21 days. The half life time of th total human protein is 80 days, that from the proteins of the liver and the plasma only 10 days. A normal adult weighing 70 kg. appears to produce a daily quantity of plasmatic and hepatic protein which corresponds to 6.2 g. nitrogen a little less than half the total protein synthesis which involves 15 3 g. nitrogen

GOLDSWORTHY and VOLWILER (1958) injected dog plasma labelled in vivo into other dogs. They found that albumin from the plasma has a shorter substitution time than other fractions. JEFFAY and WINZLER (1958) observed the same facts in rats. They also found that the quantity of protein in the diet is decisive for the duration of the substitution of albumin. The more protein contained in the diet the shorter was the half life time observed. This was not true of other protein fractions of the plasma.

ROBERTS and KELLEY (1956) added plasma proteins labelled in vitro with heavy carbon to rat liver homogenates. It could be shown that these proteins were used for energy (labelled CO_2) and glyconeogenesis. The albumin fraction was the most active one in this respect. If we summarize the results of the many experiments with labelled nitrogen-containing

nutrients, we arrive at the conclusion that FOLIN's theory is no longer completely tenable. A body in nitrogen equilibrium is not a static organism with some wear and repair of proteins. There is a dynamic equilibrium between the body proteins and many nitrogen-containing components of the diet, as the result of which the cytoplasm is continuously changed, while furthermore a regular exchange occurs between the individual tissues.

(3) *Changes in the relative quantities of the different body proteins*

CATHCART and GREEN (1913), CATHCART and BURNETT (1916) and WILSON (1925 1926, 1931 and 1932) have repeatedly observed that the proportion between nitrogen and sulphur in urine and faeces underwent a change immediately after an lteration of the diet and after other measures associated with building-up or breaking-down of tissues, while as a rule the initial N/S ratio could soon afterwards be observed again. FAY and MENDEL (1925-1926) fed dogs after a period of fasting with a normal diet in which the proportion between nitrogen and sulphur was 16.3 During the first 2 days after the change of diet the excreted matter showed N/S ratios of 41 and 36, respectively When dogs were made to fast after having been kept for some time on a calorically adequate, protein-free diet, the urine also contained relatively less sulphur during the first few days.

CUTHBERTSON (1929) observed that in human subjects with a normal nitrogen balance after immobilization of an extremity in a plaster cast, the excretion of a number of substances underwent an increase within 1 or 2 days, the excretion of sulphur being the first to increase followed by nitrogen, phosphorus and calcium, in that order The extra loss continued fairly constantly for periods of varying length, after which it decreased slowly The N/S ratio of the excreted material after application of a plaster cast indicates a source rich in sulphur and therefore in Cuthbertson's opinion it is almost certainly the consequence of break down of muscular tissue. However the proteins from muscular tissue do not contain more sulphur-containing amino-acids than most other body proteins (MÜTING and WORTMAN, 1954). According to these and other investigators (BLOCK and WEISS, 1956) the serum albumin is particularly rich in sulphur

On the basis of these tests WILSON assumed that there exist several types of depot protein, which differ in lability and in sulphur content. PETERS and VAN SLYKE (1946) argue against this view without, however advancing another theory If we consider the dynamic state of the protein metabolism it seems obvious to seek an explanation in the relations between individual body proteins. If the sulphur content of the protein produced differs from that of the disintegrated protein, the nitrogen-sulphur ratio in the excretions will be altered. In the discussion of our personal research the changes of the N/S ratio under different circumstances will be referred to again.

LUCK (1936) observed in rats that the quantity of hepatic protein increased by 1?0 when a low-protein diet was replaced by a high-protein diet whereas the muscular protein increased only 10 / by such a change of diet.

ADDIS, POO and LEW (1936 1936, 1937),

N^{15} content of protein nitrogen obtained from different organs after feeding L-leucine and glycine (25 mg N per day)
(Calculated for 100 atom per cent N^{15} in compound administered)

organ	after feeding L-leucine	after feeding glycine
serum	1 67	1 78
haemoglobin	0.29	0 46
liver	0.94	1 40
intestinal wall	1 49	0 98
kidney	1 38	—
heart	0.89	—
spleen	1 10	—
testes	0 77	—
skin	0.18	—
muscle	0.31	0.29

BORSOOK, DEASY HAAGEN SMIT KEIGHLEY and LOWY (1950) treated mice intra venously with glycine, leucine or lysine labelled with C^{14} These amino-acids disappeared from the blood within ten minutes, while within half an hour 75 / could be demonstrated in the proteins of the intestines. Expiration of labelled CO also started soon. Soon *after* the appearance of the labelled amino-acids in the abdominal organs they could also be demonstrated in the serum proteins.

GREENBERG and WINNICK (1948) carried out an investigation in rats which were treated intravenously with 25 mg. labelled glycine in order to determine the per centage of C^{14} per gramme of organic protein after fifteen minutes and after six hours respectively They found for the intestinal wall 0.3 and 3 7 for the bone marrow 0.2 and 2.15 for the liver 0 25 and 2.05 for the kidneys, 0.15 and 1.95 for the plasma, 0 05 and 1 8 for the spleen, 0 05 and 1 45 for the lung, 0 15 and 1.25 for the testes, 0 05 and 0 5 for the muscles 0.0 and 0.15 for the erythrocytes, 0 0 and 0 15 and for the brain, 0 0 and 0 1

Labelled amino-acids, incorporated in protein are replaced again by non labelled substances. From the rate at which this happens, SPRINSON and RITTENBERG (1949) have calculated the half life time of proteins The rat replaces half the total body protein in 17 days, the proteins of plasma and visceral organs in 6 or 7 days and those of the carcass in 21 days. The half-life time of th total human protein is 80 days, that from the proteins of the liver and the plasma only 10 days A normal adult weighing 70 kg. appears to produce a daily quantity of plasmatic and hepatic protein which corresponds to 6.2 g nitrogen a little less than half the total protein synthesis, which involves 15.3 g. nitrogen

GOLDSWORTHY and VOLWILER (1958) injected dog plasma labelled in vivo into other dogs. They found that albumin from the plasma has a shorter substitution time than other fractions. JEFFAY and WINZLER (1958) observed the same facts in rats. They also found that the quantity of protein in the diet is decisive for the duration of the substitution of albumin. The more protein contained in the diet the shorter was the half life time observed This was not true of other protein fractions of the plasma.

ROBERTS and KELLEY (1956) added plasma proteins labelled in vitro with heavy carbon to rat liver homogenates. It could be shown that these proteins were used for energy (labelled CO_2) and glyconeogenesis. The albumin fraction was the most active one in this respect. If we summarize the results of the many experiments with labelled nitrogen-containing

blood, while the urea concentration did not increase even when the kidneys had been removed. Similar observations have been made in cases of hepatic intoxication (STADIE and VAN SLYKE, 1920 LEWIS and IZUME, 1926–1927). Nevertheless, desamination does not take place in the liver exclusively The influence of the kidney has been described by KREBS (1935) and desamination by the intestinal wall has been demonstrated by LONDON, DUBRLSKY WASSILEWSKAJA and PROCHOROWA (1934).

KREBS (1933 1935) could demonstrate in vitro with the aid of renal tissue, that the desamination of amino-acids is based mainly on oxidation, during which keto-acids are formed, and that the reaction is accelerated by an enzyme or enzymatic system which probably differs in composition for each separate amino-acid.

BRAUNSTEIN and KRITZMANN (1937) could show that transammination, also, may take place in many different tissues. This reaction is based on the transmission of the amino-group of an amino-acid to the alpha-keto-acid of another amino-acid The dicarboxylic acids are the most active, and glutamic acid in particular COHEN (1939 1940) and COHEN and HEKHUIS (1941) were initially of the opinion that the importance of transammination was being exaggerated, but later investigations by CAMMARATA and COHEN (1950) supported the view formulated by Braunstein and Kritzmann that many amino-acid are involved in these reactions. Many transamminations could be carried out with the aid of extracts of livers, hearts and kidneys of hogs. From a review by GUNSALUS (1950) it appears that although so far only two transaminases have been investigated extensively there is sufficient evidence to render it probable that transamination is of the greatest significance for the metabolism of protein. This view is in agreement with the conclusions of Schoenheimer *et al* who observed that the dicarboxylic acids, especially glutamic acid, incorporated more isotopes than the other amino-acids, after oral administration of ammonium salts labelled with heavy nitrogen The great importance of the dicarboxylic acids for the intermediary metabolism of proteins, carbohydrates and fats appears from the close relations between the alpha-keto-acids in question and the tricarboxylic acid cycle of KREBS (1934). Through this cycle, which SOSKIN and LEVINE (1953) called "the final common pathway of metabolism frag ments of the foodstuffs may undergo many substitutions.

The carbohydrate metabolism occurs for a significant part via pyruvic acid, the alpha-keto-acid of alanine which by transamination is closely connected with glutamic acid.

KREBS and JOHNSON (1937) demonstrated that the acetyl-coenzyme A formed from fatty acids (active acetic acid), can with the aid of alpha-keto-asparaginic acid be transformed into citric acid, a part of the tricarboxylic acid cycle of Krebs

Many chemical reactions involved in the intermediary metabolism of proteins, carbohydrates and fats have been proved *to be reversible.* The differences between the influence of carbohydrates and of fats on the protein metabolism, presently to be described, have mostly been attributed to the non-reversibility of some reactions, as the consequence of which formation of

made the following observations in rats

1) after 2 days fasting the liver lost 20 / of the protein while the protein content of the rest of the body decreased 4 /

2) after 7 days fasting the quantity of liver protein had decreased by 40 / that of the kidney by 20 /, that of the blood also by 20 / and that of the carcass (muscle, skin skeleton) by 8 /

3) when casein was administered after a *low protein diet the quantity of hepatic* protein increased again rapidly at first then slower whereas the renal protein *showed a quite gradual increase.*

When dogs are subjected daily to blood letting and reinfusion of the erythrocytes after these have been washed hypoproteinaemia develops. With the aid of this plasmapheresis MADDEN GEORGE, WARAICH and WHIPPLE (1938) calculated from the duration of the endogenic substitution of plasma proteins, *that in dogs 10 to* 60 g. organic protein is kept in reserve to be used for the construction of proteins *from the blood* When dogs are rendered anaemic and are given a diet without protein and rich in iron they form 40 – 50 g. haemoglobin per week for which consequently the tissues supply the protein The urea production is very small in these animals, because the nitrogen-containing material is saved as much as possible (the high caloric value of the diets is a considerable help in this respect!) (DAFT ROBSCHEIT ROBBINS and WHIPPLE, 1933)

A sterile abscess decreases the production of *haemoglobin in a fasting,* anaemic dog (ROBSCHEIT ROBBINS and WHIPPLE, 1936) When the anaemia has been long present such an abscess exerts a still greater negative influence on the regeneration of haemoglobin (DAFT ROBSCHEIT ROBBINS and WHIPPLE, 1937).

For the explanation of a number of observations made during personal research, which will be discussed presently *we have made use of certain data mention*ed above which for this reason are stated again here

1) *Studies with isotopes have clearly* shown that substitutions in the body proteins may occur rapidly and on a large scal

2) The activity of the individual organs differs. The liver and the serum proteins *are very actively involved in the dynamics* of protein metabolism. The isotopes are incorporated in the liver *quicker than in the proteins of the serum*

3) The substitution time of the proteins from the liver and the serum is short especially when a high-protein diet is administered Albumin has *a shorter half life time than globulin*

It appears therefore that the liver is capable of quickly supplying a quantity *of protein possibly partly* at the expense of the serum albumin production.

(4) *Formation of proteins from amino-acids transamination the role of carbohydrates and fats for protein metabolism*

VAN SLYKE and MEYER (1913–1914) have demonstrated that intravenously administered alanine is rapidly incorporated in the tissues, the liver being stocked first. *Soon thereafter the urea concentration of* the blood increases. KING and RAPPORT (1933) confirmed this in the case of tyrosine.

BOLLMAN MANN and MAGATH (1926) observed that *after resection of the liver* intravenously administered amino-acids were not *quickly withdrawn from the*

significance of a number of these nitrogen compounds, among them urea, in analogous experiments in young men. SCHOENHEIMER (1946), however, observed in rats that the heavy nitrogen, with which the orally administered urea had been labelled was not incorporated in any other compound. It is possible that the influence of urea from the food depends on the presence of ammonia-forming microorganisms in the intestine, although it cannot be excluded that under special conditions the body may be able to use the nitrogen of urea for the synthesis of proteins.

The protein synthesis can be adversely influenced when an amino-acid is present in the food in an excessive quantity. CHRISTENSEN, STREICHER and ELBINGER (1948) observed in guinea-pigs that there exists a competition between the amino-acids present in the extracellular fluid where the penetration into the cells is concerned. Glutamic acid, which favours the passage through the cellular wall of other amino-acids, proved to be the only exception. DESHPANDE, HARPER and ELVEHJEM (1958) observed retardation of growth in rats when one or several amino-acids were administered in larger quantities. FISHER (1954) points out the considerable difference there may be between the metabolism of an amino-acid when it is present in excess and when it is present in a normal concentration in the food. He gives methionine as an example.

ROSE and WIXOM (1955) in healthy men could not achieve nitrogen equilibrium with high-calorie diets, in which the main source of nitrogen was essential amino-acids in twice the quantity determined by Rose as minimal. After addition of a small quantity of glycine, increasing the total quantity of nitrogen to 3½ g. per day it became possible to maintain nitrogen equilibrium. These investigators state that even a smaller quantity may suffice because the diet they administered contained approximately 1 g. of non-utilizable nitrogen.

ROSE, COON and LAMBERT (1954) revealed that the influence of protein on the nitrogen balance may be more favourable than the effect of equivalent amino-acid mixtures. When casein as the only source of nitrogen is replaced by an equal quantity of the amino-acids it contains, or by a mixture of the eight essential amino-acids, with glycine added, a greater caloric value is required if the nitrogen equilibrium is to be retained.

The biological value of a protein is high when essential and non-essential amino-acids are present in a favourable proportion and are made readily available to the organism. Taking these two factors into account, MITCHELL and HAMILTON (in DOYER, 1951) have composed a table mentioning the biological value of a number of proteins for the human subject. There is no agreement concerning the methods to be used for the determination of the biological value of proteins. As a rule it is determined how much protein must be added to diets that contain *no* protein but for the rest are optimal, in order to achieve a precise *nitrogen* equilibrium. Others are of the opinion that a more exact method is that in which a diet containing a small quantity of egg protein is enriched with a small quantity of the protein to be studied, after which the value of the protein can be assessed from the proportion between intake

carbohydrates from fats would not be possible (PETERS and VAN SLYKE, 1946) SOSKIN and LEVINE (1952) however are of the opinion that gluconeogenesis from fatty acids in diabetic subjects has clearly been demonstrated The greater importance of carbohydrates for protein metabolism can be explained by the marked tendency of glucose to take part in the final common pathway of metabolism with the chemical reactions taking place in the sense of formation of proteins and fats rather than conversely

KAPLAN and GREENBERG (1944) have treated rats intravenously with radioactive sodium phosphate. They observed that the radioactivity of adenosine triphosphate in the liver increased very rapidly when glucose had been injected shortly before the administration of the phosphate.

HERVEY and MCCANCE (1952) investigated the significance of carbohydrates in subjects who ingested carbohydrates exclusively Administration of approximately 100 g. carbohydrate caused a considerable decrease of the ketosis, a marked saving of protein and a decrease of the basal metabolism The authors regard these effects of carbohydrate as connected with the role of the tricarboxylic acid cycle for the metabolism of proteins, carbohydrates and fats.

(5) Optimal composition of proteins in the diet

ROSE (1949) subdivided the biochemical amino-acids into essential and non-essential of which the former have to be ingested with the food unless the alpha keto-acid is present in the food. This may be transformed into the amino-acid by reamination or transamination Lysine is the only amino-acid which in the (rat)body cannot be synthesized in any way (SCHOENHEIMER 1946)

The nature and proportions of the essential amino-acids differ considerably from one species of animals to another and within one species from one period of life to another Sometimes for optimal growth a higher percentage of a certain amino-acid is required than proved sufficient for the adult organism Cystine is not essential but addition of this amino-acid to the diet reduces the amount of methionine required (WOMACK and ROSE, 1941).

The synthesis of body proteins can be optimal only when all amino-acids are supplied simultaneously ELMAN (1939) 6 hours after administering an amino-acid mixture without tryptophan gave the lacking amino-acid intravenously The nitrogen balance which previously was negative then became positive MELNICK OSER and WEISS (1946) gave as their opinion that the protein of the soya bean is inferior to animal protein because the amino-acids are not absorbed in a sufficiently quick succession.

The growth and the nitrogen balance of young rats can be improved by adding ammonium salts, glutamic acid glycine or even urea to diets which contain essential amino-acids as the only sources of nitrogen (ROSE, SMITH, WOMACK and SHANE, 1949 FROST 1950) In the course of similar tests RECHCIGL, LOOSLI and WILLIAMS (1957) demonstrated that the best results are obtained with glutamic acid, followed by alanine asparaginic acid asparagine proline, glutamine di-ammonium citrate urea, biuret, glycine and serine in that order ROSE and WIXOM (1955) confirmed the

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and excretion of nitrogen (THANNHAUSER, 1957)

RIPPON (1959) studied a number of methods for the determination of the nutritional value of proteins. The results are not completely in agreement, and it does not clearly emerge which method is to be preferred.

We still have too little knowledge concerning the minimal requirements of amino-acids the optimal quantitative proportions of the amino-acids in the food and the biological value of nutritional proteins to justify exact determinations regarding the proteins fulfilling the requirements for an optimal diet.

IV Quantitative data concerning the influence of carbohydrates and fats on protein metabolism

There exists an extensive literature concerning the influence of non nitrogen containing nutrients on the protein metabolism which can be classified as follows

(1) DURING ADMINISTRATION OF A DIET WITH NORMAL PROTEIN CONTENT

a) *The influence of carbohydrates and fats as a source of energy*

It has been shown by various investigators that when carbohydrates or fats are added to diets fully adequate without them a positive nitrogen balance occurs In dogs this fact could be demonstrated by VOIT (1869), LEVENE and KOBER (1908) KOCHMANN and PETSCH (1911) LARSON and CHAIKOFF (1937), and ALLISON and ANDERSON (1945) Experiments in rats gave similar results FORBES, BRATZLER THACKER and MERCY (1939) FORBES and SWIFT (1944) LATHE and PETERS (1949) MUNRO and WIKRAMANAYAKE (1954). CUTHBERTSON and MUNRO (1937) and MUNRO and WIKRAMANAYAKE (1954) demonstrated that this saving occurs in the human as well. The last mentioned investigators observed during a 5-day period that 200 g. sucrose, daily added to an optimal diet, decreased the nitrogen excretion by some 2 g. per 24 hours. The degree of protein saving appeared to be proportional to the caloric value of the extra food (VOIT 1869 CUTHBERTSON and MUNRO 1937)

The duration of the positive nitrogen balance obtained by adding extra carbohydrates or fats to a complete diet was variable as a rule it was observed that the saving decreased after a few days, but KRUG (1894) could still demonstrate a distinct decrease of the nitrogen excretion on the 15th day when carbohydrates and fats were added daily to a complete diet. Experiments in dogs (LARSON and CHAIKOFF 1937) also proved that on the 7th day of the extra feeding there still existed a positive nitrogen balance

VOIT (1869 1869) added carbohydrate fat or a mixture of the two to a calorically insufficient protein-containing diet given to dogs and so succeeded in decreasing the nitrogen excretion JANSEN (1917) could achieve an equilibrium both in body weight and nitrogen balance by administering 125 g. carbohydrate extra to a man with severe malnutrition who in the course of World War I lived on the German people's rations, a diet yielding 1600 calories and containing 60½ g. protein Prior to the administration of the carbohydrate the average weight loss per day was 280 g. and the total nitrogen excretion exceeded the uptake by approximately 2 g. per day

RUBNER (1879) observed a definite correlation between nitrogen saving and the caloric value of the food in a healthy man who added increasing quantities of carbohydrates and fats to a protein-containing diet. When carbohydrates or fats were withdrawn from protein-containing diets the nitrogen excretion increased in healthy persons (LUSK, 1890 ROSEMANN, 1901). BOSSHARDT PAUL, O'DOHERTY and BARNES (1948) were able to confirm this on the basis of experiments in mice.

In summary many investigations have confirmed that the nitrogen balance is influenced favourably by the addition of carbohydrates or fats to protein-containing diets.

b) *Specific influence of carbohydrates and fats*

Most of the experiments concerning the comparison of the protein-saving effects of carbohydrates and fats have been carried out in fully grown persons and animals, in whose diets the carbohydrates were replaced wholly or in part by an iso-caloric quantity of fat. It is not easy to evaluate these experiments because many factors have to be taken into account, and not all investigators have always done this. MUNRO (1951) mentions some sources of error the use of inaccurate food tables, failure to determine the nitrogen in the faeces, permitting a day off between two test periods, and applying a wrong reduction factor in connection with the conception of iso-caloric. Also, the preparatory period is often too short, and changes of the non-protein nitrogen value of the blood are often neglected. Notwithstanding these shortcomings the literature does give one an impression of the difference in protein-saving significance between carbohydrates and fats.

SILWER (1937) replaced carbohydrate almost completely by fat in healthy persons on an optimal diet. In two persons whose diet contained 155 g. protein the nitrogen excretion in the urine during administration of a high-carbohydrate diet was 20.2 and 21.2 g., respectively whereas during the high-fat diet the urine contained 25.0 and 27.4 g. nitrogen, respectively At the end of the period on the high-fat diet, which lasted 11 days, no decrease of the daily nitrogen excretion was noticeable up to then.

Many investigators have iso-calorically replaced part of the carbohydrates of optimal diets by fats, which usually resulted in an increased nitrogen excretion.

RUBNER (1883), LÜTHJE (1906) and UMEDA (1915) demonstrated this in dogs. VOIT and KORKUNOFF (1895), TSUJI (1915) and UMEDA (1916), observed, also in dogs, that an increase of the quantity of protein in the diet had a more favourable influence on the nitrogen balance when the iso-caloric diets contained more carbohydrate.

UMEDA (1915) fed a healthy man first for 9 days with a diet containing 52 g. protein, 79 g. carbohydrate and 190 g. fat, and subsequently with an iso-caloric diet which contained the same quantity of protein but 413 g. carbohydrate and 43 g. fat. The average nitrogen excretion in the urine during the first period amounted to 9.27 g. per day while the high-carbohydrate diet resulted in a decrease to 6.41 g. Another man was given three different, iso-caloric diets, which contained 50 g. protein and respectively 443 g. carbohydrate and 30 g. fat, 137 g. carbohydrate and 165 g. fat, and 80 g. carbohydrate and

and excretion of nitrogen (THANNHAUSER, 1957).

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also, after a protracted period on a low protein diet, there would exist a critical caloric value above which the diet cannot bring about a further saving of protein.

This possibility has been investigated in an experiment, which will presently be reported, in a healthy man who had a low protein diet for a long time.

b) *Specific influence of carbohydrates and fats*

ZELLER (1914) administered approximately 20 g. protein per 24 hours to a healthy man, and for the rest carbohydrates exclusively. Replacement of 25 % or 50 % of the carbohydrate by fats caused a slight increase of the nitrogen excretion, and with a diet in which 75 % of the nitrogen-free food consisted of fat, a slightly more favourable result was obtained. There were, however, differences between the total caloric count and the protein value of the diets, so that the findings cannot be accepted in their entirety.

SILWER (1937) prescribed a diet to two healthy men which consisted of 20 g. protein, 280 g. carbohydrates and 129 g. fat, and this diet was replaced 11 days later by an iso-caloric diet which contained the same quantity of protein, 40 g. of carbohydrate and 235 g. fat. The excretion of nitrogen with the urine showed a considerable increase after the change of diet, but after 10 days there was hardly any difference. In the course of the last 6 days of the high-carbohydrate diet the nitrogen excretion amounted to 4.7 and 3.6 g., respectively per day. After the switch to the high-fat diet 6.0 and 5.7 g. were excreted on the average per day for the first 10 days, and thereafter for 6 days the urine contained 4.9 and 3.9 g. nitrogen per day. In these experiments, the effect of the high-fat diet on faecal loss of nitrogen was not taken into account.

With low protein diets iso-caloric substitution of part of the carbohydrates by fats probably causes only a transient increase of the protein catabolism. SCHWIMMER and MCGAVACK (1948) treated 3 groups of 4 persons with a diet of 900 calories which contained 6 g. protein and 10 %, 20 % and 30 % fat, respectively. It was observed that the nitrogen excretion was lowest with the diet that contained the most fat. Further data on this experiment are not available.

(3) DURING THE ADMINISTRATION OF A PROTEIN-FREE DIET

a) *Influence of carbohydrates and fats as sources of energy*

Most of the experiments with protein-free diets have been carried out in dogs. The excretion of nitrogen during fasting decreased distinctly after administration of carbohydrates or a mixture of carbohydrates and fats, and the saving increased as more food was administered (VOIT 1869, 1869; RUBNER, 1883; MURLIN, 1907; ÖSTERBERG and WOLF, 1907; WIMMER, 1912; COWGILL, 1923; RICHET and MINET, 1925; ALLISON and ANDERSON, 1945).

These findings have been confirmed by REID (1936, 1941) in cats and by PRZYLECKI and KARCZEWSKI (1923) in frogs. The urine of these animals, which are rather unusual for nutrition tests, contained less nitrogen during glucose infusions than previously. KRISS, FORBES and MILLER (1934) in rats saw the nitrogen excretion during fasting decrease after administration of carbohydrates.

190 g. fat. The nitrogen excretion in the urine amounted to 7 81 8 17 and 8.77 g per day respectively The faeces were not examined and in between the three periods, each of which lasted 6 days, intervals of a few days with free diet were permitted which detracts from the value of the findings. BOOTHBY SANDIFORD SANDIFORD and SLOSSE (1925) obtained approximately the same results during a similar experiment of brief duration

SCHEER CODIE and DEUEL (1947) and SCHEER, STRAUB, FIELDS, MESERVE, HENDRICK and DEUEL (1947) observed that underfed rats lost less weight when the *iso-caloric diets contained an ample quantity* of fat Additional protein saving by a larger fat component could not, however be demonstrated with calorically sufficient diets. ROSENTHAL (1952) confirmed this finding during experiments in dogs. He observed that an increase of the percentage of fat from *5 to 85* with calorically complete diets exerted hardly any influence on the nitrogen excretion *but that* the high percentage of fat caused a decrease of the nitrogen excretion with diets containing 50 or 25 % of the initial caloric count. The favourable influence of the fat was most clearly observed with low protein diets.

*Although the findings of the above-*named experiments cannot be accepted without criticism the conclusion is probably justified that iso-caloric replacement of a large part of the carbohydrates by fats when a protein-containing diet is administered leads to an increase of the protein catabolism. However *there are indications* that for optimal protein saving during a calorically insufficient (and possibly low-protein) diet fat is indispensable.

(2) DURING THE ADMINISTRATION OF A DIET WITH A LOW PROTEIN CONTENT THE SIGNIFICANCE OF PROTEIN DEPLETION

a) The influence of carbohydrates and fats as a source of energy

KEMPNER (1945) giving a diet of rice and fruit containing 20 g protein succeeded in maintaining a positive nitrogen balance for a long time the urea excretion in the urine having fallen after many weeks to 2.2 g. (containing approximately 1 1 g. nitrogen) This has been confirmed by DOLE, DAHL, COTZIAS, EDER and KREBS (1950) PESCHEL and PESCHEL (1950) and CORCORAN, TAYLOR and PAGE (1951), but WATKIN FROEB, HATCH and GUTMAN (1950) are of the opinion that the nitrogen excretion after protracted use of Kempner's rice diet is still a little above the uptake KOLFF (1952) regularly observed the important reduction in protein catabolism brought about by the rice diet.

BENDITT HUMPHREYS, WISSLER STEFFEE FRAZIER and CANNON (1948) for their experiments used adult rats which were given a very low protein diet for a period of several months Under these conditions, the nitrogen balance could not be improved by increasing the caloric value to more *than 1200 per sq metre body surface per day* while with more protein in the diet extra protein saving proved possible at a much higher caloric level. The critical value above which in case of low protein diet no improvement of the nitrogen balance *could be achieved was* the number of calories which the laboratory animals just needed for their basal metabolism and the normal daily exertions. On the basis *of these experiments* it might be expected *that in the human*

also, after a protracted period on a low-protein diet, there would exist a critical caloric value above which the diet cannot bring about a further saving of protein.

This possibility has been investigated in an experiment, which will presently be reported, in a healthy man who had a low-protein diet for a long time.

b) *Specific influence of carbohydrates and fats*

ZELLER (1914) administered approximately 20 g. protein per 24 hours to a healthy man, and for the rest carbohydrates exclusively. Replacement of 25 % or 50 % of the carbohydrate by fats caused a slight increase of the nitrogen excretion, and with a diet in which 75 % of the nitrogen-free food consisted of fat, a slightly more favourable result was obtained. There were, however, differences between the total caloric count and the protein value of the diets, so that the findings cannot be accepted in their entirety.

SILWER (1937) prescribed a diet to two healthy men, which consisted of 20 g. protein, 280 g. carbohydrates and 129 g. fat, and this diet was replaced 11 days later by an iso-caloric diet which contained the same quantity of protein, 40 g. of carbohydrate and 235 g. fat. The excretion of nitrogen with the urine showed a considerable increase after the change of diet, but after 10 days there was hardly any difference. In the course of the last 6 days of the high-carbohydrate diet the nitrogen excretion amounted to 4.7 and 3.6 g. respectively per day. After the switch to the high-fat diet 6.0 and 5.7 g. were excreted on the average per day for the first 10 days, and thereafter for 6 days the urine contained 4.9 and 3.9 g. nitrogen per day. In these experiments, the effect of the high-fat diet on faecal loss of nitrogen was not taken into account.

With low-protein diets iso-caloric substitution of part of the carbohydrates by fats probably causes only a transient increase of the protein catabolism. SCHWIMMER and MCGAVACK (1948) treated 3 groups of 4 persons with a diet of 900 calories which contained 6 g. protein and 10 %, 20 % and 30 % fat, respectively. It was observed that the nitrogen excretion was lowest with the diet that contained the most fat. Further data on this experiment are not available.

(3) DURING THE ADMINISTRATION OF A PROTEIN-FREE DIET

a) *Influence of carbohydrates and fats as sources of energy*

Most of the experiments with protein-free diets have been carried out in dogs. The excretion of nitrogen during fasting decreased distinctly after administration of carbohydrates or a mixture of carbohydrates and fats, and the saving increased as more food was administered (VOIT 1869, 1869; RUBNER, 1883; MURLIN, 1907; ÖSTERBERG and WOLF, 1907; WIMMER, 1912; COWGILL, 1923; RICHET and MINET, 1925; ALLISON and ANDERSON, 1945).

These findings have been confirmed by REID (1936, 1941) in cats and by PRZYLECKI and KARCZEWSKI (1923) in frogs. The urine of these animals, which are rather unusual for nutrition tests, contained less nitrogen during glucose infusions than previously. KRISS, FORBES and MILLER (1934) in rats saw the nitrogen excretion during fasting decrease after administration of carbohydrates.

GRÄFE (1910) demonstrated the protein saving effect of carbohydrates in humans who had previously been fasting BUTLER, TALBOT MACLACHLAN APPLETON and LINTON (1945) observed that the excretion *of nitrogen in the urine* was greater in a fasting man than in three others who ingested 50 100 and 300 g. glucose per day respectively The excretion per kg. body weight during 6 days totalled 11 3 8 3 6 4 and 6 2 g. nitrogen. It must be doubted that these figures are of significance for the degree of protein catabolism with varying quantities of sugar because *different test subjects were used and possible* changes of the rest nitrogen level of the blood were not taken into account. The latter objection can also be made in connection with a study by GAMBLE (1946–1947) in the same test person who after a 6-day period of fasting achieved a significant nitrogen saving with 100 g. of glucose per day but saw practically no decrease of the *nitrogen excretion when 6 days later the* glucose administration was increased to 200 g. per 24 hours.

The significance of the caloric value of the food for the degree of protein catabolism with protein-free diets appears from a number of observations in human persons who used such a diet for protracted periods. CATHCART (1921) found *a minimal nitrogen excretion in urine* and faeces of 2.84 g per day SMITH (1926) on *the last day of a 24 day period in which a practically protein free calorically* adequate diet was used, observed *the lowest* nitrogen excretion which amounted to 2.54 g. BOOTHBY SANDIFORD SANDIFORD and SLOSSE (1925) under similar circumstances saw a minimal excretion in urine and faeces of 1 74 g nitrogen per day which corresponds to the catabolism of less than 11 g. *protein*

BORST (1947 1948) emphasized the *great significance* of protein-free calorically adequate diets for the restriction of the protein catabolism in patients with decreased renal function. In one of the cases reported, the quantity of nitrogen excreted per day via the urine in urea and ammonium decreased to 1 4 g. per 24 hours The *significant protein-saving influence* of such diets in patients with uraemia *has been confirmed by* BULL, JOEKES *and* LOWE *(1949)*

Consequently both in animal experiments and in humans the significant role of the caloric value of the diet for protein catabolism has been repeatedly demonstrated. The findings of Butler *et al* and of Gamble, which have been quoted above are not in agreement with this conclusion However the results of these investigations cannot be accepted without criticism.

b) *Specific influence of carbohydrates and fats*

After a period of fasting the nitrogen excretion is decreased by administration of carbohydrates, whereas as a rule no saving of protein is achieved with diets that contain fats exclusively This fact has been investigated with dogs by RICHET and MINET (1925) and with rabbits by RUBNER (1883) and HEILMER (1910) THOMAS (1910) in a brief human experiment could not demonstrate any saving of protein by fat *after fasting, either*

Accordingly complete substitution of fats *for carbohydrates with protein-free diets* usually brings about a distinctly enhanced excretion of nitrogen. LUTZ and FLASCHENTRÄGER (1925) and ZUELZO (1934) were able

to demonstrate this fact in dogs, and ZAGAMI (1930) in hogs.

LANDERGREN (1903) treated a healthy man with a calorically adequate diet, which during the first 4 days contained carbohydrates exclusively and during the next 3 days only fat. The quantity of nitrogen excreted with the urine during the carbohydrate diet amounted to 8.91 5.15, 4.30 and 3.76 g. and during the fat diet 4.28, 8.86 and 9.64 g. per day. CATHCART (1922) treated a healthy man with a diet which consisted of 323 g. olive oil and subsequently replaced an iso-caloric portion of it by dextrose, 30, 60, 100 and 150 g. respectively. On the third (last) day of each period the excretion of nitrogen in the urine was 14.2, 10.2, 8.6, 7.1 and 7.4 g. respectively. ZELLER (1914) in a healthy man replaced a varying portion of a carbohydrate diet by an iso-caloric portion of fat, which led to an increased excretion of nitrogen only when more than 80 / of the carbohydrates had been replaced. This result is not completely reliable because the quantity of protein aried between 2.1 and 5.3 g. per 24 hours, while the caloric value was not completely constant either. WILLMAN, BRUSH, CLARK and SWANSON (1955) demonstrated in rats, that the nitrogen excretion increased when the fat component of iso-caloric, protein-free, low-caloric diets was decreased to less than 20%. This dependence on the presence of fat in the food for optimal protein-saving did not exist in the case of calorically sufficient diets. These results are in agreement with the previously reported observations by SCHIER *et al.* and by SCHWIMMER and McGAVACK in high-protein and low-protein diets, respectively.

It appears therefore that for protein-free diets there exist differences between the protein-saving by carbohydrates and by fats:

a) administration of fat exclusively causes no decrease of the nitrogen excretion during fasting.

b) complete replacement of carbohydrate by fat practically always causes an enhanced excretion of nitrogen.

c) partial replacement of carbohydrate by fat as a rule has no influence on the nitrogen excretion, unless the diet contains practically no carbohydrates. In a healthy man the nitrogen exeretion increased when the quantity of carbohydrate decreased to less than 100 g. per day.

d) with a protein-free, low-caloric diet, also, a given quantity of fat is probably of some significance for optimal protein-saving.

(4) INFLUENCE OF THE SIMULTANEOUS OR NON-SIMULTANEOUS CONSUMPTION OF PROTEINS AND CARBOHYDRATES OR FATS

LARSON and CHAIKOFF (1937) discovered that optimally fed dogs showed no increased nitrogen retention when they were given one additional portion of carbohydrate when it was administered long before or long after the principal meal.

CUTHBERTSON and MUNRO (1939) examined the excretion of nitrogen in four healthy men, who received 4 meals per day with various proportions of protein and carbohydrate. When the carbohydrates were divided over 2 meals and the protein over the 2 others, the daily excretion of nitrogen in the urine was approximately 2 g. more than when both substances were equally distributed over the 4 meals. Shift

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(5) CONCLUSIONS CONCERNING THE PROTEIN-SAVING EFFECT OF CARBOHYDRATES AND FATS

The principal data concerning the protein-saving effect of carbohydrates and fats can be summarized as follows

1) It has been demonstrated in laboratory animals as well as in human beings, that addition of carbohydrates or fats to *high-protein* diets causes a saving of nitrogen, the degree of which is proportional to the caloric value of the nitrogen-free nutrients administered in addition. When a large part of the carbohydrates in a protein-containing diet is replaced by fat the excretion of nitrogen increases. When the diet is insufficient from a caloric point of view fat is probably indispensable for optimal protein-saving.

2) Under favourable circumstances, nitrogen equilibrium can be achieved in the human with calorically sufficient diets which contain approximately 20 g. protein. It has been demonstrated in animal experiments that in a condition of protein depletion, there exists for *low-protein* diets a critical caloric value above which no improvement of the nitrogen balance can be obtained this value concerned the precise number of calories required for the basal metabolism and the normal daily exertion. The iso-caloric substitution of fat for a large part of the carbohydrates temporarily decreases the saving of protein.

3) It has been shown in animal experiments that the nitrogen excretion with *protein-free* diets is proportional to the caloric value of the nutrition, provided the diet contains a small amount of carbohydrates In the human, this quantity of carbohydrates amounts to 100 g. per 24 hours. In apparent contradiction to the animal experiments, BUTLER *et al* and GAMBLE observed in the human that the nitrogen excretion did not decrease when, with a diet consisting exclusively of sugar the quantity of sugar was increased to more than 100 g. per day This contradiction will be further discussed during the report of personal experiments.

The protein-saving effect of carbohydrates can be divided into a number of factors (MUNRO, 1951)

a) based on the caloric value, iso-calorically replaceable by fat and independent of administration simultaneously with protein

b) especially as regards nutritional protein, not replaceable by fat and depending upon administration simultaneously with protein

c) especially as regards body-protein, partually replaceable by fat.

V Protein metabolism and sodium chloride

TERROINE and MAHLER MANDLER (1927) saw that the excretion of nitrogen in rats increased when sodium chloride was withdrawn from the diet. SANDERSON (1954) studied the excretion of electrolytes in patients with ulcer of the stomach, who were treated with continuous intravenous administration of sodium bicarbonate. This caused a distinct retention of sodium and a slight retention of chloride, whereas the potassium balance remained practically unaltered. In one of the patients who for 13 days received 1000 m.eq (84 g.) $NaHCO_3$, the urea clearance increased, the blood urea concentration decreased and the urea excretion decreased also but to a lesser degree After the treatment had

of 25 g. carbohydrate to the protein-containing carbohydrate free meals brought about the same protein-saving as 100 g. In one experiment it could be shown that *for optimal protein-saving* it was not necessary for all protein to be administered together with the carbohydrate. When two *meals contained not only* carbohydrate and fat but also a small quantity of protein and the two other meals consisted of meat exclusively no additional protein saving could be achieved by consuming part of the carbohydrates together with the meat The results of the changes of diet were always examined for only a few days

Munro (1949) demonstrated in rats that for maximal protein-saving carbohydrates had to be administered together with, soon before or soon after the protein This time factor was not valid in the case *of fat, even though in connection with the* slower resorption the fat was administered 1½ to 2½ hours before the protein The nitrogen loss brought about by the separate administration of protein and carbohydrate was compensated by an extra retention after the change-back to the initial mixed meals. Evidently the lost *depot protein* was supplemented again by the improved method of feeding.

Geiger Bancroft and Hagerty (1950) observed in rats *that* the *nitrogen-excretion* was smaller the sooner carbohydrate was administered after a protein *meal whereas the same could not* be demonstrated for fat This time linked protein-saving influence could be observed *for a few days* only in normal adult rats, but the effect persisted for a longer time in growing rats and in *adult animals which* had too small a stock of protein

Munro and Wikramanayake (1954) in optimally fed dogs, demonstrated that the protein saving caused by *one additional* carbohydrate meal was the greater the sooner this meal was administered after the protein-containing basic meal but at the first repetition of this daily extra *feeding the time-factor had lost its influence* The same investigators gave to 4 optimally fed men 200 g. of sucrose extra, *divided over the usual meals or 5½ hours* after the last meal With the first method the nitrogen excretion decreased that same day whereas administration of carbohydrate after the meals caused a (somewhat slighter) protein-saving only after 2 days.

In other words, carbohydrates apparently have a protein-saving effect, which depends on *administration at the same* time as protein and of which the following particulars have been observed

a) *when extra carbohydrates are added* daily to an optimal diet the time of administration of the carbohydrates has completely or almost completely lost its influence as early as the second day

b) the time-factor retains its influence for a longer time when a positive nitrogen balance can be maintained

c) for optimal saving *it is not necessary* that all the protein be administered together with the carbohydrate nor is it necessary for all carbohydrates to be added to the nutritional protein

It has been demonstrated in laboratory animals that this influence of the time of administration *does not exist in the case* of fat. The effect of fat in this respect has not been studied in man This aspect will be discussed in a personal experiment to be reported presently

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2) Under favourable circumstances, nitrogen equilibrium can be achieved in the human with calorically sufficient diets which contain approximately 20 g. protein. It has been demonstrated in animal experiments that in a condition of protein depletion, there exists for *low-protein* diets a critical caloric value, above which no improvement of the nitrogen balance can be obtained this value concerned the precise number of calories required for the basal metabolism and the normal daily exertion The iso-caloric substitution of fat for a large part of the carbohydrates temporarily decreases the saving of protein.

3) It has been shown in animal experiment that the nitrogen excretion with *protein-free* diets is proportional to the caloric value of the nutrition provided the diet contains a small amount of carbohydrates. In the human, this quantity of carbohydrates amounts to 100 g. per 24 hours. In apparent contradiction to the animal experiments, BUTLER *et al* and GAMBLE observed in the human that the nitrogen excretion did not decrease when, with a diet consisting exclusively of sugar the quantity of sugar was increased to more than 100 g. per day This contradiction will be further discussed during the report of personal experiments.

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been discontinued, the converse phenomena were observed Apparently these alterations were the consequence of changes of the glomerular filtration and of an influence of the protein synthesis.

LEAF and COUTER (1949) treated healthy persons first with a low-salt diet for a week, then for 3 days with 480 m eq sodium chloride per day This caused a reduction in the output of nitrogen but the excretion of phosphorus and of potassium was not always affected accordingly The continuation of this salt administration led to an increased excretion of potassium.

In a later discussion of personal experiments the importance of sodium chloride for the metabolism of protein will be considered

VI Protein metabolism and bed rest

SPENCE, EVANS and FORBES (1946) were able to prove that during strict bed rest nitrogen equilibrium can only be obtained if the diet is rich in protein KEYS (1944) observed nitrogen equilibrium during administration of a diet containing 54 g of protein to a subject who performed his normal activities. During a 3-week period of bed rest this diet led to a loss of protein of 280 g. after the quantity of protein in the diet had been increased to 110 g. per day the nitrogen excretion became equal to the intake

The catabolic influence of immobilization on the metabolism of protein also emerges clearly from the previously described observations of CUTHBERTSON (1929), who in healthy persons had a plaster cast applied to one of the lower extremities after which an increased excretion of protein metabolites in the urine and faeces could soon be demonstrated.

The significance of bed rest for protein metabolism was often clearly proved by our personal experiments, to which reference will be made later

VII. Application of data from nutrition physiology to the treatment of patients with renal failure

(1) *Caloric value*

In the work of BORST (1947 1948), previously mentioned, attention was called for the first time to the significance of the caloric value of the diet for the restriction of protein catabolism in patients with low renal function This was later confirmed by BULL, JOEKES and LOWE (1949). MERRILL (1960) quotes Borst's work on the basis of which he assumes that in the long run additional protein-saving is obtained by high-caloric diets (after a period of 20 days or longer), but that initially no saving of nitrogen must be expected when the quantity of sugar in the diet is increased to more than 100 g. per 24 hours. Merrill bases his opinion upon the investigation by Gamble which we have earlier referred to in which after a 6 days period of fasting, a marked saving of protein was observed when 100 g. glucose was given per 24 hours, whereas 6 days later no decrease of the protein catabolism could be demonstrated after the quantity of sugar in the diet had been increased to 200 g. In view of this same observation MERRILL (1955) is of the opinion that the diet during acute anuria does not have to contain more than 100 g. of sugar unless, as is usually the case, the protein catabolism is enhanced as the consequence of stress, infection trauma or surgery

These considerations of Merrill are based on phenomena observed by Gamble in a single person. We therefore did a further investigation of the protein-saving significance of various quantities of sugar with protein-free diets (see experiments IV and V discussed below)

There are no theoretical objections against giving a large part of the calories in the form of fat. Optimal protein synthesis is only possible however if the diet contains at least 100 g. of carbohydrates.

There is evidence that for optimal protein-saving with a calorically insufficient diet fat is indispensable. Its significance was greatest with low-protein diets.

If we want to make use of the specific saving of nutritional protein by carbohydrate, it is desirable that at least part of the proteins be administered together with carbohydrate. The quantitative protein-saving significance of the simultaneous administration of protein and carbohydrate will be discussed during an experiment reported below (IX).

It is possible that the less protein there is in the diet, the smaller is the critical caloric value in excess of which practically no further protein-saving can be achieved. In this connection it appears improbable that the protein catabolism can be restricted to a significant degree by increasing the number of calories beyond that necessary for maintenance of the body-weight. This aspect will be further investigated in experiment III

( ) *Quantity of protein in the diet*

There are fundamentally different views concerning the quantity of protein which the diet must contain in cases of chronic uraemia. Many authors are of the opinion that at least 40 g. of protein must be given per day (0.5 g. protein per kg. body weight), whereas others regard the quantity of protein in the diet as dependent upon renal function, and sometimes allow only 20 g. per day or even less.

It appears from the data and the literature that it is possible to obtain nitrogen equilibrium with diets that contain approximately 20 g. protein. Obviously a diet with which under favourable conditions nitrogen equilibrium can barely be maintained can be accepted only when absolutely necessary It can be readily understood therefore that many authors think that more protein must be allowed to patients with chronic uraemia, because they fear a severe protein deficiency (PETERS and VAN SLYKE, 1946 LIPPMAN and PERSIKE, 1947 FISHBERG, 1954 MERRILL, 1955 1960).

It seems unjustified to reduce the protein intake too strictly in an endeavour to lower the blood urea concentration in patients with severe renal failure to less than 600–900 mg./litre, but diets which contain 40 g. protein in the later stages of chronic nephritis often cause a much higher blood urea concentration, frequently associated with nausea, vomiting and itching. In such cases, the disadvantages of th high percentages of retained protein catabolites probably outweigh the advantage of the slightly greater protein synthesis that can be obtained with diets containing as much as ½ g. protein per kg. body weight. BORST (1948) as a rule prescribes for patients with renal function of less than 10 / a calorically adequate diet containing approximately 20 g. protein per day There is no absolute proof that this

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The catabolic *influence of immobilization* on the metabolism of protein also *emerges clearly from the* previously described observations of CUTHBERTSON (1929) who in healthy persons had a plaster cast applied to one of the lower extremities, after which an increased excretion of protein metabolites in the urine and faeces could soon be demonstrated.

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(1) *Caloric value*

In the work of BORST (1947 1948), previously mentioned attention was called for the first time to the significance of the caloric value of the diet for the restriction of protein catabolism in patients with low renal function This was later confirmed by BULL, JOEKES and LOWE (1949). MERRILL (1960) quotes Borst s work on the basis of which he assumes that in the long run additional protein-saving is obtained by high-caloric diets (after a period of 20 days or longer) but that initially no saving of nitrogen must be expected when the quantity of sugar in the diet is increased to more than 100 g per 24 hours. Merrill bases his opinion upon the investigation by Gamble, which we have earlier referred to in which after a 6 days period of fasting, a marked saving of protein was observed when 100 g. glucose was given per 24 hours, whereas 6 days later no decrease of the protein catabolism could be demonstrated after the quantity of sugar in the diet had been increased to 200 g. In view of this same observation MERRILL (1955) is of the opinion that the diet during acute anuria does not have to contain more than 100 g of sugar unless as is usually the case the protein catabolism is enhanced as the consequence of stress, infection trauma or surgery

These considerations of Merrill are based on phenomena observed by Gamble in a single person. We therefore did a further investigation of the protein-saving significance of various quantities of sugar with protein-free diets (see experiments IV and V discussed below).

There are no theoretical objections against giving a large part of the calories in the form of fat. Optimal protein synthesis is only possible however if the diet contains at least 100 g. of carbohydrates.

There is evidence that for optimal protein-saving with a calorically insufficient diet fat is indispensable. Its significance was greatest with low-protein diets.

If we want to make use of the specific saving of nutritional protein by carbohydrate it is desirable that at least part of the proteins be administered together with carbohydrate. The quantitative protein-saving significance of the simultaneous administration of protein and carbohydrate will be discussed during an experiment reported below (IX).

It is possible that the less protein there is in the diet, the smaller is the critical calorie value in excess of which practically no further protein-saving can be achieved. In this connection it appears improbable that the protein catabolism can be restricted to a significant degree by increasing the number of calories beyond that necessary for maintenance of the body-weight. This aspect will be further investigated in experiment III.

( ) *Quantity of protein in the diet*

There are 2 fundamentally different views concerning the quantity of protein which the diet must contain in cases of chronic uraemia. Many authors are of the opinion that at least 40 g. of protein must be given per day (0.5 g. protein per kg. body-weight) whereas others regard the quantity of protein in the diet as dependent upon renal function and sometimes allow only 20 g. per day or even less.

It appears from the data and the literature that it is possible to obtain nitrogen equilibrium with diets that contain approximately 20 g. protein. Obviously a diet with which under favourable conditions nitrogen equilibrium can barely be maintained, can be accepted only when absolutely necessary. It can be readily understood therefore that many authors think that more protein must be allowed to patients with chronic uraemia, because they fear a severe protein deficiency (PETERS and VAN SLYKE, 1946; LIPPMAN and PERSIKE, 1947; FEINBERG, 1954; MERRILL, 1955, 1960).

It seems unjustified to reduce the protein intake too strictly in an endeavour to lower the blood urea concentration in patients with severe renal failure to less than 600–900 mg./litre, but diets which contain 40 g. protein in the later stages of chronic nephritis often cause a much higher blood urea concentration, frequently associated with nausea, vomiting and itching. In such cases, the disadvantages of the high percentages of retained protein catabolites probably outweigh the advantage of the slightly greater protein synthesis that can be obtained with diets containing as much as ½ g. protein per kg. body-weight. BORST (1948) as a rule prescribes for patients with renal function of less than 10% a calorically adequate diet containing approximately 20 g. protein per day. There is no absolute proof that this

been discontinued the converse phenomena were observed Apparently these alterations were the consequence of changes of the glomerular filtration and of an influence of the protein synthesis

LEAF and COUTER (1949) treated healthy persons first with a low-salt diet for a week then for 3 days with 480 m eq sodium chloride per day This caused a reduction in the output of nitrogen, but the excretion of phosphorus and of potassium was not always affected accordingly The continuation of this salt-administration led to an increased excretion of potassium.

In a later discussion of personal experiments, the importance of sodium chloride for the metabolism of protein will be considered

VI Protein metabolism and bed rest

SPENCE, EVANS and FORBES (1946) were able to prove that during strict bed rest nitrogen equilibrium can only be obtained if the diet is rich in protein KEYS (1944) observed nitrogen equilibrium during administration of a diet containing 54 g. of protein to a subject who performed his normal activities. During a 3-week period of bed rest this diet led to a loss of protein of 280 g. after the quantity of protein in the diet had been increased to 110 g. per day the nitrogen excretion became equal to the intake.

The catabolic influence of immobilization on the metabolism of protein also emerges clearly from the previously described observations of CUTHBERTSON (1929), who in healthy persons had a plaster cast applied to one of the lower extremities, after which an increased excretion of protein metabolites in the urine and faeces could soon be demonstrated

The significance of bed rest for protein metabolism was often clearly proved by our personal experiments, to which reference will be made later

VII Application of data from nutrition physiology to the treatment of patients with renal failure

(1) Caloric value

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with the same dose again showed the same influence on the protein synthesis.

Important differences have been found in the protein-anabolic effect of various androgenic steroids. The nitrogen excretion can also be decisively influenced by the dosage, the duration of the treatment and even by the rate of absorption. One of the principal differences between the anabolic steroids concerns the distribution of the retained protein over the organs. Although there are convincing arguments against making a fundamental distinction between the androgenic and the anabolic effect of the steroids in question (KASSENAAR, 1952), it is of practical significance to know whether a saving of nitrogen can be obtained with doses which have little or no virilizing effect. WAINMAN and SHIPOUNOFF (1941) demonstrated that in rats the perineal striated musculature shows a considerable increase in size under the influence of TP. Although it is fundamentally incorrect to regard the growth of these muscles as a non-androgenic effect, it appears that the ratio between the weight gain of the seminal vesicles and the levator ani muscle after application of the steroid to be studied in castrated rats, constitutes a good estimate of the virilizing influence to be expected.

(2) *Effect of androgens in subjects receiving normal amounts of protein*

KOCHAKIAN and MURLIN (1935) found that in castrated dogs no increase of the protein-anabolic effect of androgens could be obtained by increasing the percentage of protein in the diet to above normal. KOCHAKIAN and VAN DER MARK (1952) observed in castrated rats that the saving of nitrogen after administration of TP was not altered when the percentage of protein in the diet was increased from 18 to 28 or to 43 %. KENYON and KNOWLTON (1946) observed a decrease of the protein-saving effect of TP when the caloric value and the quantity of nitrogen of the diet of a healthy man were decreased from 2816 to 2251 and to 1589 and from 13.5 g. to 4.6 and to 1.7 g., respectively. FORSYTH and PLOUGH (1955), in 3 patients with cirrhosis of the liver, Reiter's syndrome and rheumatoid spondylitis, respectively and in a reasonable nutritional condition administered a diet which kept the body-weight and the nitrogen-balance just stable. Addition of 250 g. carbohydrate for 15, 16 and 18 days respectively caused a continuous decrease of the daily excretion of nitrogen of 1.0 to 1.7 mg. per additionally administered calorie which corresponds to approximately 8 g. protein per day. About the same protein synthesis could be achieved by injection of 25 mg. TP daily during the basic diet, but this treatment had not the slightest protein-saving effect during the period of extra feeding. BASSETT, KEUTMANN and KOCHAKIAN (1946), in a woman suffering from the Cushing syndrome observed that addition of carbohydrate during a maximal protein-saving effect of androgens may decrease the excretion of nitrogen.

FORSYTH (1954) examined the protein anabolic effect of TP in 4 soldiers, who had been wounded in Korea and suffered badly from malnutrition. Increase of the caloric value of the diet from 2600 to 3300 and of the quantity of protein from 97 to 199 g. per day caused no alteration of the protein-saving effect of 25 mg. TP which was administered daily during a period of

very low protein diet has more advantages than disadvantages in comparison with the diet containing at least 40 g. protein per day because an investigation with two comparable groops of patients has never been carried out. However there are some indications that a low protein diet is to be preferred. Patients, who previously were given a larger protein ration often show considerable improvement after the protein intake is limited to 20 g. per day and the urea concentration of the blood which formerly was more than 1500 mg. per litre decreases to less than 800 mg. Frequently the nausea and the apathy disappear and the appetite improves. When the protein portion of the diet is increased, these phenomena of illness return Borst thinks it likely that owing to this diet patients survive longer and remain longer able to do their work. It is no exception for patients with a renal function (creatinine clearance) of less than 10 / of the normal average to lead a normal and active life for several years. Patients who adhere strictly to the diet seldom die before the renal function has decreased to 3 %. In many cases, this function is 2 / or less during the terminal few weeks.

In view of the fact that this diet is given to many patients with chronic nephritis, we have selected a quantity of 20 g. protein in the diet for most of the experiments subsequently to be reported

VIII Effect of androgenic steroids

(1) *General remarks wearing-off' effect*

The first findings concerning the influence of androgenic substance on the metabolism of protein have been reported in 1935 by Kochakian and Murlin These authors observed that extracts from the urine of young men caused a retention of nitrogen in castrated dogs which was much greater than could be explained by synthesis of protein in the secondary sex organs. The maximal gain amounted to 0 05 g. nitrogen per kg. body-weight per 24 hours.

Soon after the chemically pure androgenic steroids became available, they were administered to patients with hypogonadism (Kenyon 1938) Later investigations have been conducted into the protein-saving effect of androgens in normal persons (Kenyon Knowlton Sandiford Koch and Lotwin 1940). Daily administration of 25 mg. testosterone propionate (TP) for a week in a normal man caused a maximum nitrogen retention of 0 03 g. per kg. body-weight per day which corresponds to a synthesis of a little more than 13 g. protein per day The result in patients with hypogonadism was about twice as great. The effect on protein-anabolism was not enhanced by increasing the dose of TP

In 1946, Kochakian summarized numerous data from the literature in an outstanding review

Kochakian (1944) observed in castrated rats that the saving of protein in spite of continued administration of TP decreased after about one week and thereafter gradually disappeared altogether This 'wearing-off' effect was not correlated with the nature of the food Bassett Keutmann and Kochakian (1943) demonstrated the same effect in a girl aged 15 years, who suffered from a Cushing syndrome, and who received 25 mg. TP per day The protein saving effect decreased after approximately one month but after a 3 months interval treatment

protein. Carbohydrates cause a decrease of the protein-saving effect of androgens after a brief period of fasting, whereas an increase of the caloric value of a calorically adequate diet has no influence on the nitrogen saving brought about by TP. When the nitrogen balance is already positive, it is only after malnutrition that a protein-saving effect of androgenic steroids can be observed.

The degree of protein-saving by androgenic substances apparently increases with the degree of protein catabolism and the protein requirements of the body (protein depletion). When a change of the diet exerts contradictory influences on these two factors, the effect of this change on the degree of protein-saving cannot be predicted.

(4) *Changes in the relative quantities of different body proteins induced by androgens: renotrophic effect*

KOCHAKIAN, COHN, QUIGLEY and TRYBALSKI (1948) observed that the prostate and the seminal vesicles of rats under the influence of TP still increased in weight when the nitrogen balance was no longer positive because of the protracted administration of the androgen. Under these conditions, therefore, protein in the body is shifted to those organs which are most influenced by the androgenic steroids.

There have been more observations of such shifts. ABELS, NELSON, YOUNG and TAYLOR (1944) treated a healthy man who was on a normal diet, with 90 mg. testosterone daily for 48 days. In the course of the first 30 days the injections caused a protein synthesis of 392 g., while the quantities of albumin and globulin in the blood plasma, determined with the aid of the plasma volume, showed a distinct decrease. During the subsequent 18 days, with continued treatment, 112 g. protein was retained: the circulating protein increasing to over the initial value. Similar but shorter-lasting experiments have been carried out in 4 other persons, 3 of whom suffered from carcinoma of the stomach.

COOPER, RYNEARSON, MACCARTY and POWER (1951) administered TP in doses between 25 and 100 mg. per day to patients with severe lesions of the spinal cord. Although the nitrogen balance improved, initially the serum protein concentration was constantly found to decrease: this decrease proved reversible after 30–60 days.

SCHAPIRA and DREYFUS (1949) cut one of the sciatic nerves in male rats. After treatment with TP the atrophy of the paralysed gastrocnemius muscle as compared with the normal muscle proved to be more pronounced than in control animals that had not been given the TP injections.

Also in the fasting organism a maximal anabolic effect is obtained in the secondary sex organs after the use of androgenic steroids. The kidneys, also may increase in size, although the effect is slighter than in animals which are fed (KOCHAKIAN 1946). This renotrophic effect of androgenic substances is described for the first time in 1934 by KORENCHEVSKY and DENNISON, who observed that the weight of the kidneys of male rats after castration decreased relatively more markedly than the body weight, a phenomenon that could be prevented by administration of extracts of the urine of men.

SELYE (1939) demonstrated in mice

8 to 14 days. The additional nitrogen retention caused by TP which also has been observed in cases where the nitrogen balance was already positive amounted to 16 to 53 mg. per kg body-weight per day COOPER, RYNEARSON, MACCARTY and POWER (1951) in patients with transverse lesions of the spinal cord observed no additional protein retention as the effect of TP when the nitrogen balance was already positive However the nutritional condition of these patients was better than that of the Korean soldiers described by Forsyth.

VAN WAYJEN, GROEN and WILLEBRANDS (1958 1959) demonstrated nitrogen retention induced with TP in a number of patients who had been subjected to total gastrectomy

(3) *Effect of androgens in subjects on low protein diets the influence of protein depletion*

GEIGER and RAWI (1952) demonstrated that administration of TP to normal rats *kept under protein free diets caused no* increase of the body weight WIJNANS and DE GROOT (1953) did observe nitrogen-saving in castrated rats, when TP was administered at the beginning of a protein free diet, whereas the androgen caused no decrease of the nitrogen excretion in rats which had been kept on a protein free diet for several weeks previously

PERLMAN and CASSIDY (1953) were unable to demonstrate any protein-saving influence of TP in castrated dogs kept on a low protein diet, but after increase of the quantity of *protein in the diet* TP caused a pronounced protein synthesis.

BARTLETT and STEVENSON (1954) on the 2nd to the 6th day inclusive, of a 6-day period of fasting treated normal femal dogs with an injection of 50 mg. TP and also on the 2nd day with an intravenous administration of glycine labelled with heavy nitrogen. During the first 24 hours after administration of glycine a greater excretion of heavy nitrogen in the urine was found than during a control period without administration of TP but the excretion of the isotope was distinctly lower during the next 4 days of the experiment. Consequently the synthesis of protein during fasting can be stimulated by androgenic steroids. The enhanced nitrogen excretion during the first 24 hours after administration of a large dose of TP has been described several times previously ARONOFF GRAHAM and MCINTOSH (1954) observed it in rats.

BUTLER, TALBOT MACLACHLAN APPLETON and LINTON (1945) in a healthy young man during a 6-day period of fasting with 25 mg. TP per day found an excretion of nitrogen in the urine which was 33 mg. per kg. body weight less than *during a control period without administration* of androgen. The protein saving effect was about half as great in 3 other healthy men, who received 50 100 and 300 g. glucose as their only food.

The interpretation of the data in the literature concerning the influence of the food on the protein saving effect of androgens is not simple. Protein synthesis during fasting can be stimulated with androgenic steroids, but the effect decreases the longer the fasting is continued *An increase* of the quantity of protein in the diet causes an increase of the nitrogen saving by androgenic substances, except when the diet already contained sufficient

(5) Methylandrostenediol nor-testosterone derivatives

Methylandrostenediol (MAD) was initially regarded as highly important because of its relatively slight androgenic action (GORDAN, EISENBERG and MOON 1950 HOMBURGER, FORBES and DESJARDINS, 1950 GORDAN, EISENBERG, MOON and SAKAMOTO, 1951 KASSENAAR, 1952). PARTRIDGE, BOLING, DEWIND, MARGEN and KINSELL (1953), however could demonstrate in 3 patients that the nitrogen-saving caused by MAD is less than that caused by TP while the larger doses of MAD whose anabolic effect was good, had a virilizing effect that was relatively as marked as that of TP

HERSHBERGER, SHIPLEY and MEYER (1953) mentioned the relatively slight virilizing effect of nor-testosterone derivatives, which differ from testosterone by the absence of the methyl group, of which the 19th carbon atom constitutes part. The role of two of these substances, viz. ethylnor-testosterone (norethandrolon, ANT) and nor-testosterone phenylpropionate (nor-androstenolone phenylpropionate NAPP) has been described by various investigators.

Ethylnor testosterone has not only a pronounced anabolic and a relatively slight androgenic effect, it has also a progestative influence. The substance has a protein-anabolic effect which appears rapidly and lasts briefly it can be administered orally SAUNDERS (1957) in castrated rats observed that the ratio of the weight gain of the seminal vesicles and the levator ani muscle was most favourable after administration of a small dose of the steroid. PRUNTY BROOKS, CLAYTON and MCSWINEY (1958) studied a boy aged 5 years, who was treated for fragilitas ossium with 20 mg. ethylnor testosterone per day for 7 weeks, and who developed a slight enlargement of the penis. The usual dose for adults is 50 mg. per day

OVERBEEK and DE VISSER (1957) were able to show in castrated rats that the proportion of the growth-promoting effect on the seminal vesicles and on the levator ani muscle was 8 times more favourable after administration of NAPP than after administration of the same dose of testosterone phenyl propionate.

The two nor testosterone derivatives exert a favourable influence especially on *a calcium balance*. VAN DONWELEN *(1956)* gave one dose of 50 mg. NAPP to a woman with carcinoma of the breast and skeletal metastases and subsequently noticed a pronounced decrease of the calcium concentration in the urine. GERBRANDY and HELLENDOORN (1957) confirmed the retention of calcium after NAPP in patients with carcinoma of the breast and osteolytic metastases the virilizing effect was relatively much slighter than after administration of TP

KOWALEWSKI and GOUWS (1957) and KOWALEWSKI (1958) observed in rats that a fractured bone incorporated a higher percentage of intraperitoneally administered radioactive sulphur when the animals had been treated with ethylnor testosterone whereas pretreatment with TP had no influence. The *sulphur is used for the* formation of chondroitine-sulphuric acid which is indispensable for fracture healing (DZIEWIATKOWSKI, 1951).

The data from the literature concerning the protein-anabolic effect of the nor testosterone derivatives are as a rule less

that androgenic steroids can cause enlargement of the kidneys in normal animals as well In a later publication (1941) SELYE showed that the structure of the renal tubules in mice changes after orchiectomy an effect that can be undone by androgenic steroids.

KASSENAAR (1952) observed that the renotrophic effect of androgens in castrated mice depends on the increase of the intra cellular fluid the protein percentage of the renal tissue being initially diminished He also observed swelling of the tubular cells and a decrease of the diameter of the lumen

FRIEDEN LABY BATTS and LAYMAN (1957) and FRIEDEN and COHEN (1958) have proved that the homogenates of mouse kidney to which has been added glycine labelled with heavy nitrogen incorporated more isotopes when the mice had been treated with TP whereas the uptake of homogenates of the liver the diaphragm and the skeletal muscles was independent of the administration of TP

WELSH ROSENTHAL, DUNCAN and TAYLOR (1942) found that in dogs the administration of TP was not followed by improvement of the renal functions, apart from a distinct increase of the maximal tubular excretion for diodrast. LATTIMER (1942) noticed that TP after unilateral nephrectomy in dogs and in rats caused an accelerated compensatory hypertrophy of the kidney He also described the cases of two patients, in whom nephrectomy had to be carried out because of hypernephroma and one of whom was treated from the ninth postoperative day with 25 mg. TP per day for fourteen days. The renal functions of these two patients were the same with the exception of the Tm for diodrast which was greater in the man treated with TP However Lattimer could not demonstrate the slightest improvement of tubular function after administration of TP in normal persons, in 3 patients who had been subjected to nephrectomy one year previously and in 2 patients with chronic nephritis with hypertension. KLOPP YOUNG and TAYLOR (1944) and DEAN ABELS and TAYLOR (1945) gave testosterone and TP in various doses to healthy persons and to patients with disturbances of the renal function but never obtained any improvement of function.

FREEDMAN and SPENCER (1957) determined the nitrogen balance and the urea clearance in 10 patients with insufficient renal function and an increased urea concentration of the blood. In view of the protein anabolic effect and the improvement of renal function obserbed in experiments in animals, the patients were treated with TP in doses of 50 mg. per day for 14 days. The diet constantly contained 31 g. protein and supplied 1750 calories per day The urea clearance was not altered in any of these patients, but 8 of them showed an improvement of the nitrogen balance, corresponding to a protein synthesis of an average of 216 g in 14 days, and the blood urea concentration decreased from 1460 to 920 mg. per l. Two cases failed to improve at all one probably as the consequence of cellulitis and the other for no detectable reason The failure of TP in the case with cellulitis is compared by the authors with a study by MASSON CORCORAN and PAGE (1949), who with the aid of androgen were able to prolong the life of rats after bilateral nephrectomy except when a turpentine abscess had been provoked

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convincing than the data concerning the retention of calcium. There have been some favourable reports concerning the use of NAPP in surgical patients (HARTENBACH 1957) and in tropical asthenia (LAMBILLON 1957) but no exact data concerning the nitrogen balance have been added JONXIS and MAATS (1957) saw a definite weight gain in children who had been treated with NAPP but they also state that the appetite improved so that the children more often ate their meals completely

NOWAKOWSKI and PARADA (1958) in two patients with endocrine osteoporosis observed that administration of a single dose of 100 mg. NAPP was followed not only by a pronounced calcium retention but also by an improvement of the nitrogen balance which persisted for 18 days at least.

A number of preliminary reports have been published concerning the use of nor testosterone derivatives in patients with affections of the kidneys. MCCRACKEN and PARSONS (1958) in 6 patients with an acute disturbance of renal function after an obstetrical affection observed a decrease of urea production after administration of ethylnor testosterone in doses of 80 mg per 24 hours for 5 days. In 5 similar non obstetrical patients and in 5 healthy persons the substance had no protein saving influence at all It is to be regretted that the scarcity of data in this publication renders evaluation of the results impossible.

GJÖRUP and HESS THAYSEN (1958) obtained favourable results with ANT and NAPP in 10 patients with severe chronic uraemia. It appears from a nitrogen balance that 40 to 100 mg. ANT per day caused a decrease of the blood-urea concentration in a patient with a creatinine clearance of less than 5 ml. per minute, in spite of a diet that contained 50 to 75 g. protein per day Evaluation of this nitrogen balance is made difficult by the fact that the diet was altered daily and that no data are presented concerning the calorie value of the diets.

From the data in the literature concerning the protein-anabolic effect of androgenic steroids the following conclusions can be drawn

1) In certain hormonal dysfunctions a considerable protein synthesis can be achieved Examples are hypogonadism, various forms of endocrine osteoporosis, endocrine dwarfism and the Cushing syndrome.
2) Less certain is the quantitative significance of the protein synthesis caused by androgenic steroids in patients losing protein for no demonstrable hormonal cause e g after burns, after fractures, during infectious diseases and in the postoperative periods.
3) When anabolic steroids are indicated in women or children the nor testosterone derivatives are to be preferred ethylnor testosterone is sometimes contraindicated because of its progestative action
4) There have been some preliminary reports concerning stimulation of protein synthesis by nor testosterone derivatives in patients with affections of the kidneys, which render further investigation desirable.

C

PRESENT INVESTIGATION

I. Concise description of 16 balance tests methods

We shall successively describe a number of balance experiments after which we shall consider whether conclusions may be drawn from combinations of observations.

The nitrogen balance and the excretion of total nitrogen and several protein catabolites were studied during the following experiments

I Administration of a diet that consisted only of 600 g. sugar and 1800 ml tap water per day Subject man aged 42. Duration of the experiment 22 days.

II Administration of diets of adequate caloric value, containing 22½ and 137 g. protein per day respectively In this experiment the influence of certain androgenic substances was also investigated. Subject same man aged 45 Total duration of the experiment 141 days.

III Administration of diets which contained approximately 20 g. protein and yielded 500, 1000, 1500 2000, 2500, 3000 and 3500 calories per day respectively a study was also made of the protein-saving effect of an increase of the quantity of salt in the diet during 3 days and of 3 anabolic steroids. Subject same man aged 48. Total duration of the experiment 156 days.

IV Administration of a calorically sufficient diet containing 22 g. protein per day the influence of certain nor testosterone derivatives on the synthesis of protein was studied. Following this diet the protein catabolism was studied during diets which consisted exclusively of water and 100 and 300 g. sugar per day respectively Subject woman aged 45. Total duration of the experiment 99 days.

V Administration of diets, consisting exclusively of water and 100 and 400 g. sugar per day respectively following the administration of calorically adequate diets, containing 80 g. protein per day Subjects 2 men, both aged 19 Duration of the experiment 20 days.

VI. Administration of a calorically adequate diet, which contained 80 g. protein per day During 4 days part of the protein was replaced by methionine. Subject man aged 21 Duration of the experiment 16 days.

VII. Administration of a calorically adequate fatfree diet which contained 80 g. protein per day During part of the experiment protein and carbohydrate were not given in the same meal. Subject man aged 23 Duration of the experiment 19 days.

VIII. The same experiment, using fat instead of carbohydrate Subject man

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I Administration of a diet that consisted only of 600 g. sugar and 1800 ml. tap water per day Subject man aged 42. Duration of the experiment 22 days.

II Administration of diets of adequate caloric value, containing 22½ and 137 g. protein per day respectively in this experiment the influence of certain androgenic substances was also investigated. Subject same man aged 45 Total duration of the experiment 141 days.

III. Administration of diets which contained approximately 20 g. protein and yielded 500, 1000, 1500, 2000, 2500, 3000 and 3500 calories per day respectively a study was also made of the protein-saving effect of an increase of the quantity of salt in the diet during 3 days and of 3 anabolic steroids. Subject same man aged 45. Total duration of the experiment 156 days.

IV Administration of a calorically sufficient diet containing 22 g. protein per day the influence of certain nor-testosterone derivatives on the synthesis of protein was studied. Following this diet the protein catabolism was studied during diets which consisted exclusively of water and 100 and 300 g. sugar per day respectively Subject woman aged 45 Total duration of the experiment 99 days.

V Administration of diets, consisting exclusively of water and 100 and 400 g. sugar per day respectively following the administration of calorically adequate diets, containing 80 g. protein per day Subjects 2 men, both aged 19 Duration of the experiment 20 days.

VI Administration of a calorically adequate diet, which contained 80 g. protein per day During 4 days part of the protein was replaced by methionine Subject man aged 21 Duration of the experiment 16 days.

VII Administration of a calorically adequate fatfree diet which contained 80 g. protein per day During part of the experiment protein and carbohydrate were not given in the same meal. Subject man aged 23 Duration of the experiment 19 days.

VIII The same experiment, using fat instead of carbohydrate. Subject man

convincing than the data concerning the retention of calcium. There have been some favourable reports concerning the use of NAPP in surgical patients (HARTENBACH 1957) and in tropical asthenia (LAMBILLON 1957) but no exact data concerning the nitrogen balance have been added. JONXIS and MAATS (1957) saw a definite weight gain in children who had been treated with NAPP but they also state that the appetite improved so that the children more often ate their meals completely.

NOWAKOWSKI and PARADA (1958) in two patients with endocrine osteoporosis observed that administration of a single dose of 100 mg. NAPP was followed not only by a pronounced calcium retention but also by an improvement of the nitrogen balance which persisted for 18 days at least.

A number of preliminary reports have been published concerning the use of nor testosterone derivatives in patients with affections of the kidneys. MCCRACKEN and PARSONS (1958) in 6 patients with an acute disturbance of renal function after an obstetrical affection observed a decrease of urea production after administration of ethylnor testosterone in doses of 80 mg. per 24 hours for 5 days. In 5 similar non-obstetrical patients and in 5 healthy persons the substance had no protein saving influence at all. It is to be regretted that the scarcity of data in this publication renders evaluation of the results impossible.

GJÖRUP and HESS THAYSEN (1958) obtained favourable results with ANT and NAPP in 10 patients with severe chronic uraemia. It appears from a nitrogen balance that 40 to 100 mg. ANT per day caused a decrease of the blood-urea concentration in a patient with a creatinine-clearance of less than 5 ml. per minute, in spite of a diet that contained 50 to 75 g. protein per day. Evaluation of this nitrogen balance is made difficult by the fact that the diet was altered daily and that no data are presented concerning the caloric value of the diets.

From the data in the literature concerning the protein anabolic effect of androgenic steroids the following conclusions can be drawn:

1) In certain hormonal dysfunctions a considerable protein synthesis can be achieved. Examples are hypogonadism, various forms of endocrine osteoporosis, endocrine dwarfism and the Cushing syndrome.
2) Less certain is the quantitative significance of the protein synthesis caused by androgenic steroids in patients losing protein for no demonstrable hormonal cause e.g. after burns, after fractures, during infectious diseases and in the postoperative periods.
3) When anabolic steroids are indicated in women or children, the nor testosterone derivatives are to be preferred; ethylnor-testosterone is sometimes contraindicated because of its progestative action.
4) There have been some preliminary reports concerning stimulation of protein synthesis by nor testosterone derivatives in patients with affections of the kidneys, which render further investigation desirable.

C

PRESENT INVESTIGATION

I. Concise description of 16 balance tests methods

We shall successively describe a number of balance experiments after which we shall consider whether conclusions may be drawn from combinations of observations.

The nitrogen balance and the excretion of total nitrogen and several protein catabolites were studied during the following experiments

I Administration of a diet that consisted only of 600 g. sugar and 1800 ml. tap water per day Subject man aged 42. Duration of the experiment 22 days.

II. Administration of diets of adequate calorie value containing 22½ and 137 g. protein per day respectively in this experiment the influence of certain androgenic substances was also investigated Subject same man aged 45 Total duration of the experiment 141 days.

III Administration of diets which contained approximately 20 g. protein and yielded 500 1000, 1500 2000 2500 3000 and 3500 calories per day respectively a study was also made of the protein-saving effect of an increase of the quantity of salt in the diet during 3 days and of 3 anabolic steroids. Subject same man aged 48. Total duration of the experiment 156 days.

IV Administration of a calorically sufficient diet containing 22 g. protein per day the influence of certain nor testosterone derivatives on the synthesis of protein was studied. Following this diet the protein catabolism was studied during diets which consisted exclusively of water and 100 and 300 g. sugar per day respectively Subject woman aged 45 Total duration of the experiment 99 days.

V Administration of diets, consisting exclusively of water and 100 and 400 g. sugar per day respectively following the administration of calorically adequate diets, containing 80 g. protein per day Subjects 2 men, both aged 19 Duration of the experiment 70 days.

VI. Administration of a calorically adequate diet, which contained 80 g. protein per day During 4 days part of the protein was replaced by methionine. Subject man aged 21 Duration of the experiment 16 days.

VII Administration of a calorically adequate fatfree diet which contained 80 g. protein per day During part of the experiment protein and carbohydrate were not given in the same meal. Subject man aged 23 Duration of the experiment 19 days.

VIII The same experiment, using fat instead of carbohydrate. Subject man

aged 22. Duration of the experiment 7 days.

IX Administration of a calorically adequate diet containing 20 g. protein per day During part of the experiment protein and carbohydrate or protein and fat were given separately Subject man aged 22. Duration of the experiment 49 days

X. Determination of the protein-saving brought about by methylandrostenediol (MAD) during the administration of a calorically adequate diet containing 80 g. protein per day Subject man aged 20 Duration of the experiment 20 days.

XI Similar experiment with 20 g protein per day in the diet. Subject man aged 21 Duration of the experiment 20 days.

XII Determination of the protein saving brought about by testosterone propionate (TP) during the administration of a calorically adequate diet containing 80 g. protein per day Subject man aged 19 Duration of the experiment 20 days.

XIII Similar experiment with 20 g. protein per day in the diet. Subject man aged 21 Duration of the experiment 20 days.

XIV Determination of the protein-saving brought about by nor androstenolone phenylpropionate (NAPP) and of addition of sodium chloride during the administration of a calorically adequate diet containing 20 g. protein per day Subject man aged 20 Duration of the experiment 25 days.

XV and XVI Administration of calorically adequate diets containing 18 and 15 g. protein per day respectively Determination of the anabolic effect of NAPP Subject man aged 32, suffering from severe renal failure. Duration of the experiments 45 and 33 days respectively

Methods

The body weight was determined every day after the first miction before breakfast.

The urine was studied in 3-hour 24-hour or 72 hour portions, depending on the nature of the experiment. The urine and faeces were always immediately placed in the refrigerator No preservatives were added The faeces were homogenized with the aid of an electrical apparatus

All methods of determination have been critically studied by the biochemist, L. A. de Vries. All these methods, with the exception of the determination of sulphur have been accurately described by him in GORTER and DE GRAAFF 1955

The following substances have been determined both in the urine and in the faeces

1) *Nitrogen* The total nitrogen content of the urine and the faeces was determined by the method of Kjeldahl.

2) *Sulphur* The method used for the determination of the total quantity of sulphur in the urine and faeces is a combination of the methods of BENEDICT (1909) and DENNIS and REED (1926). The faeces were incinerated with the aid of $Cu(NO_3)_2$ and $KClO_3$. The residue was dissolved in hydrochloric acid, after which a mixture of $BaCl_2$ and gelatine was added The $BaSO_4$ formed was subsequently determined nephelometrically

This method gave good results when a quantity of urine was used that contained

between 0.1 and 0.2 mg. sulphur The findings were too low when the determinations were carried out in larger quantities of urine. Addition of 1 ml. urine containing approximately 1 mg. sulphur to a solution of ammonium sulphate caused a significant decrease of the value found in the ammonium sulphate alone. This disturbing influence has been previously described by BAILEY (1937).

3) *Phosphorus* The quantity of phosphate was determined colorimetrically with the aid of molybdenic acid.

4) *Potassium* and *sodium* were determined by flame photometry

5) *Calcium* was determined colorimetrically with the aid of complexon

Furthermore the following substances were determined in the urine

1) *Urea plus ammonia* by the method of Ambard.

2) *Ammonia* by the method of Ronchese and Malfatl.

3) *Creatinine* by the reaction of Jaffe as modified by De Vries and Van Daatselaar

Also several determinations were carried out in the blood

1) *Urea*, with the urease method.

2) *Haemoglobin* colorimetrically by the alkaline haematine method.

3) *Blood plasma protein fractions* by the salting-out method of Majoor

The results of every experiment ha e been recorded in a table and drawn into a summarizing graph the nitrogen, sulphur phosphorus and potassium balances have sometimes been reproduced on a larger scale according to the method of REIFENSTEIN ALBRIGHT and WELLS (1945). The

Diagram to illustrate the balance of one constituent
The intake is plotted downwards from the base line
The output is plotted upwards from the bottom of the intake
The faecal excretion is charted beneath as a cross-hatched area.
(Reifenstein, Albright and Wells, 1945)

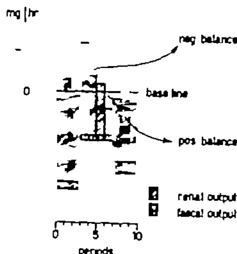


Fig. 1

principle of this method is shown in fig. 1 From the horizontal base line the intake of the substance studied is recorded in a downward direction. Starting from the lowest level of the intake, the faecal excretion is drawn in the upward direction, the renal excretion being shown above it. When the base line is not reached by the excretion, the balance is positive If the excretion extends above the base line, the balance is negative

When the balance of two substances are shown in one figure, for purposes of comparison, different scales have been used, corresponding to the proportion in which the two substances are present in muscular protoplasm. This ratio is 14½ for nitrogen and sulphur 15 for nitrogen and phosphor

aged 22. Duration of the experiment 7 days.

IX. Administration of a calorically adequate diet containing 20 g. protein per day During part of the experiment protein and carbohydrate or protein and fat were given separately Subject man aged 22. Duration of the experiment 49 days.

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XV and XVI Administration of calorically adequate diets containing 18 and 15 g. protein per day respectively Determination of the anabolic effect of NAPP Subject man aged 32, suffering from severe renal failure Duration of the experiments 45 and 33 days respectively

Methods

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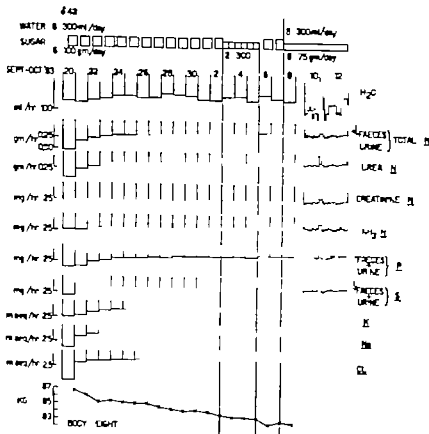


Fig 3 Exp I Excretion of protein catabolites during administration of diet consisting exclusively of 600 g. sugar daily for 22 days. Bed rest during last 3 days.

Consequently the diet contained approximately 2.3 m.eq. sodium, 0.1 m.eq potassium, 8.3 m.eq. calcium and 3.0 m.eq chloride per day

Vitamins were administered in the form of a commercially available aqueous solution (Dohyfral multi liquidum, N.V Philips-Roxane, Weesp). This preparation prescribed in a daily dose of 2 × ½ ml., contains per ml. 5000 I U vitamin A, 1.8 mg. vitamin B I 0.8 mg. vitamin B II, 1 mg. vitamin B VI 15 mg. nicotinic acid amide 1.5 mg. d panthenol, 7.5 mg. aminobenzoic acid, 50 mg. vitamin C and 5000 I.U vitamin D III. These quantities of vitamins are on the whole in reasonable agreement with the requirements of an adult subject (Nederlandse Voedingsmiddelentabel, 18th ed., 1958), although the quantity of vitamin B I is possibly insufficient with a diet containing carbohydrates exclusively It is, however improbable that the protein synthesis during this experiment which lasted 22 days, was influenced by a deficiency of one of the vitamins.

During the first 13 days the sugar and water were divided into 6 equal portions,

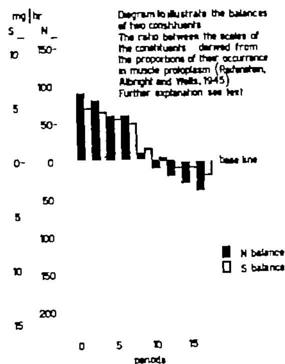


Fig. 2

us, and for nitrogen (in mg) and potassium (in m.eq) 370 (REIFENSTEIN ALBRIGHT and WELLS, 1945) In order to facilitate comparison the balances were not superim posed but placed alongside each other from day to day An example is shown in fig 2.

In most of the experiments the diet consisted of milk protein sugar and butter so that the determination of the composition gave no difficulties. In two cases a complete study has been made of a more variegated diet, and in 3 cases determinations from a table have been used (Nederlandse Voedingsmiddelentabel 1958).

The following preparations have been examined for their protein-saving effect

1) Testosterone propionate (Neohombreol, Organon) A crystalline substance dissolved in oil which is absorbed relatively rapidly and has a fairly rapid short lasting action (abbreviated TP).

2) Testosterone phenylpropionate (TPP Organon). A substance dissolved in oil with a moderately retarded action (TPP).

3) Methylandrostenediol (Neosteron, Organon) An aqueous suspension with delayed absorption (abbreviated MAD)

4) 19-Nor-androstenolone phenylpropionate (Durabolin Organon). A steroid dissolved in oil with prolonged action (abbreviated NAPP)

5) 19 Nor-androstenolone decanoate. An ester dissolved in oil with a markedly prolonged action (abbreviated NAD)

6) Ethylnor testosterone (Nilevar Searle). Tablets with quick absorption (abbreviated ANT)

7) Androstadienolone. This substance has been shown to have a nitrogen-saving effect in rats. In view of later experiences it is not used therapeutically

II. Discussion of the results of the experiments

I – THE FIRST EXPERIMENT was carried out in a healthy man aged 42, who for 22 days received only 600 g. sugar and 1800 ml tap water daily No salts were added to this diet.

The tap water in Amsterdam originates from various sources so that the composition differs from one part of the town to another As a rule water from the southern or from the central part of the town was drunk. Chemical examination of these two types of tap water gave the following results (in m.eq per litre)

	sodium	*potassium*	*calcium*	*chloride*
Amsterdam (South)	1 01	0 056	4.25	1.24
Amsterdam (Centre)	1.62	0.073	5 07	2.13

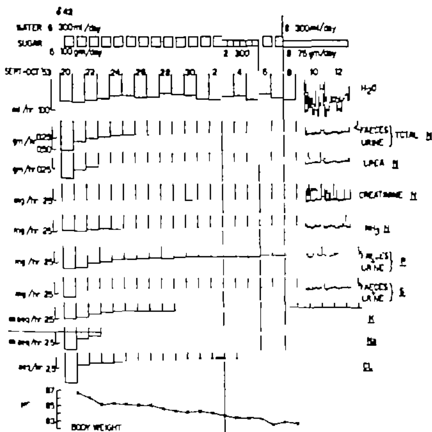


Fig 3 Exp 1 Excretion of protein catabolites during administration of diet consisting exclusively of 600 g. sugar daily for 22 days. Bed rest during last 3 days.

Consequently the diet contained approximately 2.3 m.eq sodium 0.1 m.eq potassium, 8.3 m.eq calcium and 3.0 m.eq chloride per day

Vitamins were administered in the form of a commercially available aqueous solution (Dohyfral multi liquidum, N V Philips-Roxane Weesp). This preparation prescribed in a daily dose of 2 × ½ ml., contains per ml. 5000 I U vitamin A, 1.8 mg. vitamin B I 0.8 mg. vitamin B II 1 mg. vitamin B VI 15 mg nicotinic acid amide 1.5 mg. d-panthenol, 7.5 mg. aminobenzoic acid 50 mg. vitamin C and 5000 I U vitamin D III. These quantities of vitamins are on the whole in reasonable agreement with the requirements of an adult subject (Nederlandse Voedingsmiddelentabel, 18th ed., 1958), although the quantity of vitamin B I is possibly insufficient with a diet containing carbohydrates exclusively It is, however improbable, that the protein synthesis during this experiment which lasted 22 days, was influenced by a deficiency of one of the vitamins.

During the first 13 days the sugar and water were divided into 6 equal portions,

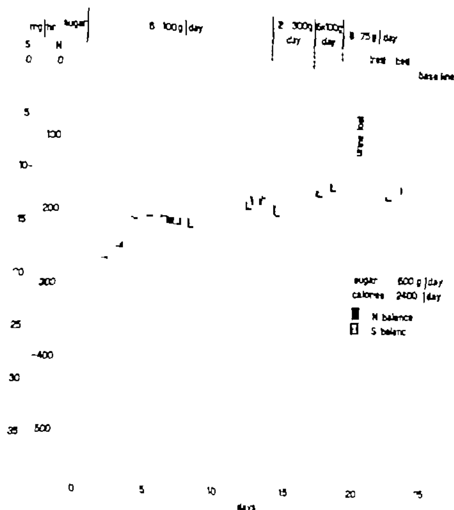


Fig. 4 – Exp 1 N and S balance during administration of diet consisting exclusively of 600 g. sugar daily for 2_ days.

which were ingested at 8 11 a.m. and 2, 5 8 and 11 p m during the next 3 days the water was given at the same times, but the sugar was taken in 2 portions of 300 g at 8 a.m and 8 p m. After this period the sugar was again divided into 6 equal portions for 3 more days.

During these 19 days the subject performed his normal activities as a physician and thereafter kept strict bed rest for 3 days. During these last 3 days he ate 75 g. sugar every 3 hours (also totalling 600 g) together with 300 ml water (totalling *2400 ml*).

The urine was collected every 24 hours except during the period of bed rest when every 3-hour portion was examined With a view to the examination of this rhythmic excretion the quantity of water in the diet was increased.

The following determinations were carried out body weight, volume of urine, nitrogen sulphur phosphorus, urea, creatinine ammonia, potassium, sodium and chloride in the urine, and nitrogen sulphur and phosphorus in the faeces.

The findings are shown in table I and in figure 3 Some of the data have been set forth separately on a larger scale

(1) From the excretion curves for the

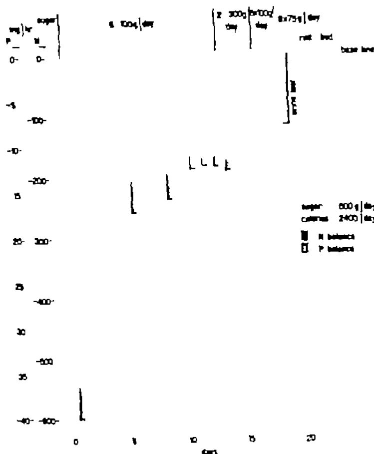


Fig. 5 E 1 N and P balance during administration of diet consisting exclusively of 600 g. sugar daily for 22 days.

protein catabolites it appears that the excretion decreased considerably during the first few days and subsequently underwent a gradual, but much slower further decrease. From the review of the literature it appears that observations of this sort constitute a strong indication of the existence of a quantity of body protein (depot protein), which is catabolized rapidly under circumstances of deficiency. On the last (19th) day of the first period the smallest total nitrogen excretion was observed viz. 3.3 g., corresponding to approximately 20.6 g. protein. Optimal conditions for protein-saving did not exist during this experiment. As stated in the review of the literature a deficiency of sodium chloride has an unfavourable influence on protein anabolism.

(2) When we desire to investigate the influence of the diet on the function of the kidney as an excretory organ, we must remember that the proportions of nitrogen, phosphorus, sulphur and potassium excreted with the urine vary partially as a consequence of the composition of the diet.

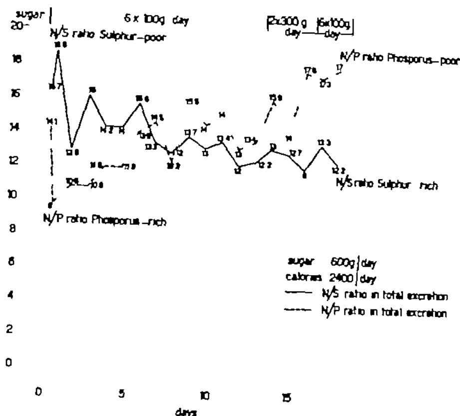


Fig. 6 – Exp. I – *Ratio of total N and S, respectively total N and P excretion during administration of diet consisting exclusively of 600 g. sugar daily for 22 days.*

For nitrogen (derived from urea and ammonia) this quantity *amounted to 74 g. in 18 days. During anuria this* would mean that the blood urea concentration would have increased in this period by approximately 2350 mg per litre During the last few days approximately 2.4 g. nitrogen was excreted per 24 hours deriving from urea and ammonia

In the case of anuria the blood urea concentration in this last period would have increased by only 77 mg. per litre per 24 hours.

The constant portion of the sulphur with the urine was not determined separately The total quantity of sulphur obtained *in 18 days* through the kidney *amounted to 6.2 g* while during the last *few days approximately 250 mg.* sulphur per *24 hours were excreted* with the urine.

The total renal excretion of phosphorus amounted to 6 3 g over *18* days, while during the last few days the urine contained approximately 150 mg. phosphorus per 24 hours.

Potassium 350 m.eq were excreted through the kidneys in 18 days, whereas during the last few days the excretion in the urine was approximately 8 m eq per 24 hours.

(3) Although the proportion of the excretion of the protein catabolites is approximately in agreement with the composition of protoplasm, it appears from figs. 4 and 5 that during the first few days relatively less sulphur and more phosphorus than nitrogen were excreted, whereas thereafter the converse phenomenon was observed This is shown in fig. 6 in a different manner

The significance of these ratios will be discussed in a separate chapter

(4) When the sugar was consumed in 2 portions of 300 g. the excretion of the protein catabolites increased The large sugar meals caused no glycosuria. The protein-saving effect of 6 × 100 g. sugar was therefore greater than that of 2 × 300 g. No great practical significance must be attached to this observation. It can be calculated from the difference in nitrogen excretion that approximately 3 g. protein was saved additionally per 24 hours when the portions were given at 4-hour intervals. Still it is to be recommended for patients with anuria e.g. that use be made several times per day of the rapid protein anabolic effect specific for carbohydrates.

(5) During the period of bed rest the excretion of the protein catabolites was found to have considerably increased again to approximately 4½ g. per 24 hours. This may have been the consequence of a better excretion due to increased diuresis as the subject ingested more fluid, but a more important factor was probably the influence of the inactivity atrophy (compare the review of the literature).

(6) It has been known for a long time that the excretion of water urea, sodium, chloride and potassium during a 24-hour period follows a certain rhythm, with more retention during the night than in the daytime but further discussion of this phenomenon is outside the scope of this publication

It was noted, however that the rhythm of excretion of phosphorus through the kidney is the opposite, more phosphorus being retained in the daytime (fig. 3 Oct. 10, 11 and 12, 1953).

(7) Although the excretion of creatinine with the urine was fairly constant, there was nevertheless a slight decrease to be observed, which was probably the consequence of a slight decrease of the quantity of muscular tissue in the course of the experiment. This finding will be discussed more extensively below

(8) The excretion of ammonia through the kidneys showed a considerable decrease during the experiment. Apparently during the catabolic processes in the body less and less acid valencies were being freed the decrease of the excretion of phosphorus and sulphur is in agreement with this.

(9) The sodium and chloride excretion initially showed a very rapid decrease. Within a few days time, the excretion of sodium was equal to the intake via the tap water The chloride balance on the other hand, was still negative after 19 days.

(10) The faeces consisted of a dark brown mucus, which was sharp and irritating so that regularly during the last 10 days there was an enhanced peristalsis and even painful tenesmus. Notwithstanding this the faeces contained only 0.24 g. nitrogen per 24 hours, although many investigators believe that 1.3 g. per day is excreted by the intestines. The significance of the intestinal mucosa for the faecal excretion of nitrogen is, therefore, presumably slight.

II THE SECOND EXPERIMENT was carried out in the same man, who was 45 years old at the time of the experiment. During the first 90 days he was given a diet which contained 22½ g. protein, 320 g. carbohydrate 200 g. fat and 12½ g. alcohol, 3190 calories altogether for 51 days immediately afterward the diet contained the

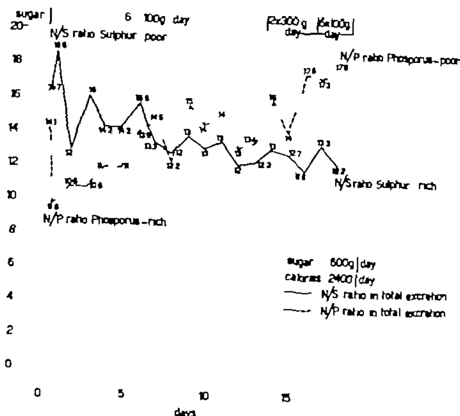


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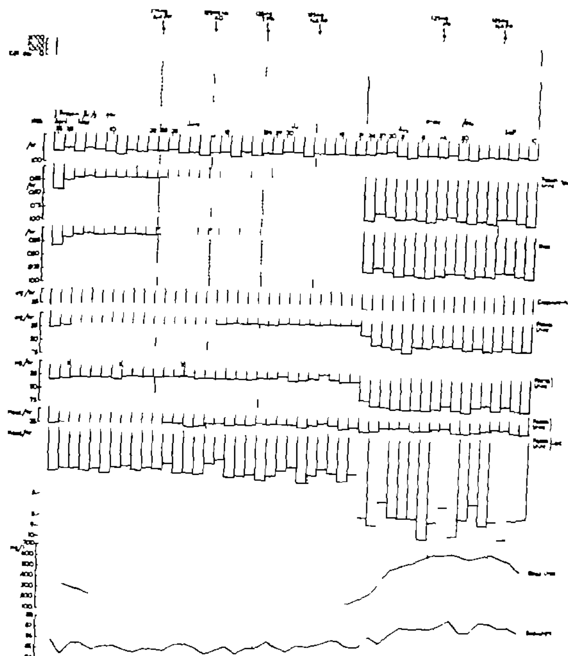


Fig. 7 – Exp. II – Excretion of protein catabolites during administration of diets with adequate caloric value: 221 g. protein for 90 days and 137 g. protein for 51 days, respectively

following daily quantities 137 g. protein 222 g. carbohydrate 185 g. fat and 12½ g. alcohol also yielding 3190 calories Water was drunk daily in equal quantities.

The diets were not analysed The composition was calculated with the aid of the Nederlandse Voedingsmiddelen Tabel (1958). During the two parts of the experiment, the same meal was eaten every third day so that 3-day periods could be compared For this reason the determinations were carried out in 72 hour specimens of urine, and the faeces were also studied The factors examined were the body-

weight, the *quantity of urine* the total excretion of nitrogen sulphur phosphorus, potassium and sodium, and the urinary ex cretion of urea and creatinine further more, the urea concentration, the haemoglobin value of the blood and the protein fractions of the bloodplasma were de termined every few days.

During this experiment, also the subject continued his normal activities as a physician. Care was taken that the amount of physical exertion was the same each day as far as possibl..

Thirty days after the beginning of the low-protein diet 125 mg. nor testosterone phenylpropionat (NAPP) was injected intramuscularly 15 days later 125 g. methylandrostenediol (MAD), 15 days after that, 125 mg. testosterone phenylpropionate (TPP) and again 15 days later another 125 mg. NAPP Fifteen days after this last injection, the patient was switched over to the high-protein diet, and he re ceived 125 mg. TPP after 21 days, and 125 mg. NAPP after 39 days.

The findings are shown in table II and in figure 7

(1) It appears from fig. 8, that after 30 days, there was still a negative nitrogen balance, which showed no tendency to improve. The excretion of nitrogen was then approximately 34 mg. per hour more than the intake so that 5 1 g. protein per day was lost. It is possible that the practically constant excretion after the 9th day of the experiment was due to a coincidental protein-catabolic action e.g. a nonma ifest focus of inflammation. As a rule it takes longer for the nitrogen excretion to become constant after an important change of the quantity of protein in the

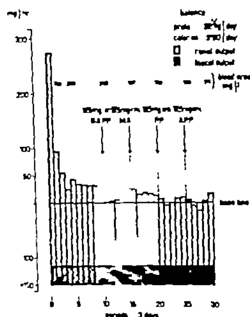


Fig. 8 Exp II Effect of anabolic steroids on the N balance during administration of diet of adequate caloric value containing 22 g. protein per day

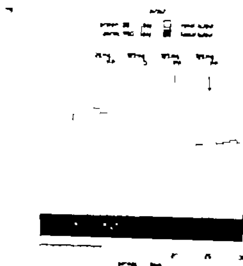


Fig. 9 E II Effect of anabol. steroids on the excretion of S during administration of diet of adequate caloric value containing 22 g. protein per day

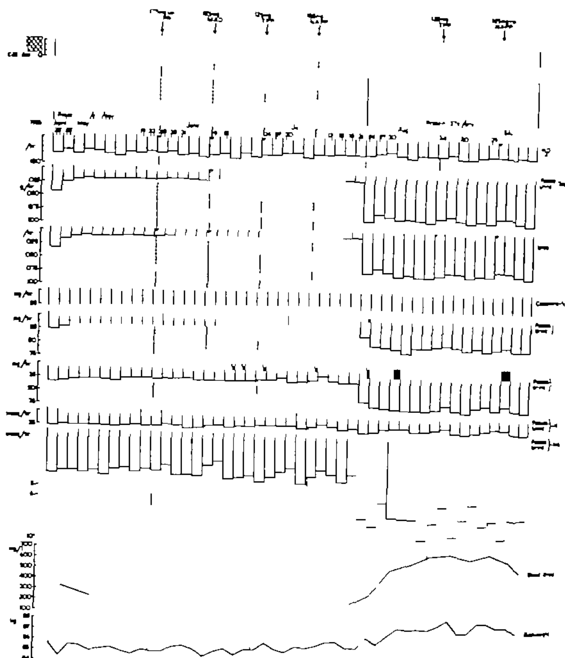


Fig 7 – Exp II – Excretion of protein catabolites during administration of diets with adequate caloric value 22½ g. protein for 90 days and 137 g. protein for 51 days, respectively

following daily quantities 137 g protein 222 g carbohydrate 185 g. fat and 12½ g. alcohol, also yielding 3190 calories. Water was drunk daily in equal quantities.

The diets were not analysed The composition was calculated with the aid of the Nederlandse Voedingsmiddelen Tabel, (1958) During the two parts of the experiment, the same meal was eaten every third day so that 3-day periods could b compared. For this reason the determinations were carried out in 72 hour specimens of urine and the faeces were also studied. The factors examined were the body

weight, the quantity of urine, the total excretion of nitrogen, sulphur phosphorus, potassium and sodium, and the urinary excretion of urea and creatinine furthermore, the urea concentration, the haemoglobin value of the blood and the protein fractions of the bloodplasma were determined every few days.

During this experiment, also the subject continued his normal activities as a physician. Care was taken that the amount of physical exertion was the same each day as far as possible.

Thirty days after the beginning of the low-protein diet 125 mg. nor testosterone phenylpropionate (NAPP) was injected intramuscularly 15 days later 125 g. methylandrostenediol (MAD), 15 days after that, 125 mg testosterone phenylpropionate (TPP) and again 15 days later another 125 mg. NAPP Fifteen days after this last injection, the patient was switched over to the high-protein diet, and he received 125 mg. TPP after 21 days, and 125 mg. NAPP after 39 days.

The findings are shown in table II and in figure 7

(1) It appears from fig. 8 that after 30 days, there was still a negative nitrogen balance, which showed no tendency to improve. The excretion of nitrogen was then approximately 34 mg. per hour more than the intake so that 5.1 g. protein per day was lost. It is possible that the practically constant excretion after the 9th day of the experiment was due to a coincidental protein-catabolic action, e.g. a nonmanifest focus of inflammation. As a rule it takes longer for the nitrogen excretion to become constant after an important change of the quantity of protein in the

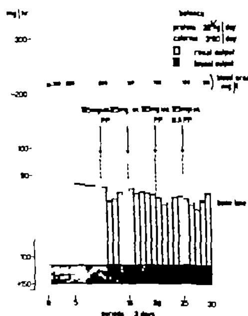


Fig. 8 Exp II Effect of anabolic steroids on the N balance during administration of diet of adequate caloric value containing 22½ g. protein per day

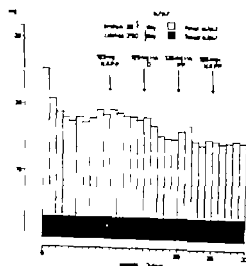


Fig 9 Exp II Effect of anabolic steroids on the excretion of N during administration of diet of adequate caloric value containing 22½ g. protein per day

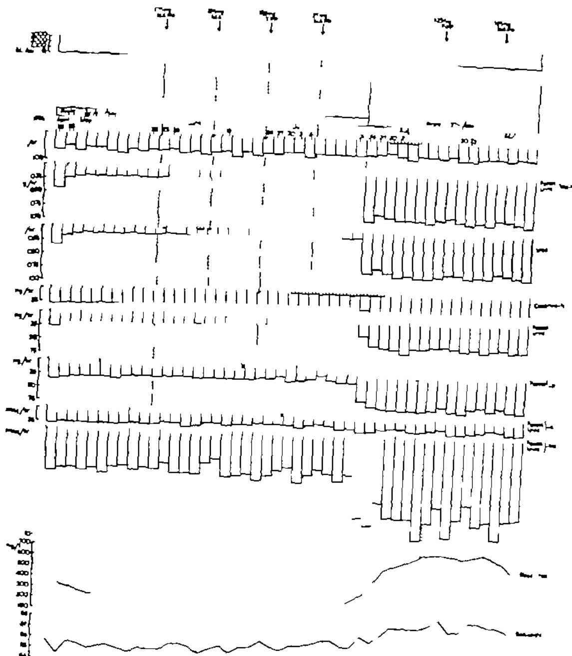


Fig. 7 – Exp II – Excretion of protein catabolites during administration of diets with adequate caloric value 22½ g. protein for 90 days and 137 g. protein for 51 days, respectively

following daily quantities 137 g. protein 222 g. carbohydrate 185 g. fat and 12½ g. alcohol, also yielding 3190 calories. Water was drunk daily in equal quantities

The diets were not analysed. The composition was calculated with the aid of the *Nederlandse Voedingsmiddelen Tabel* (1958) During the two parts of the experiment, the same meal was eaten every third day so that 3-day periods could be compared For this reason the determinations were carried out in 72 hour specimens of urine and the faeces were also studied. *The factors examined were the body*

Fig. 12 Exp. II Effect of anabolic steroids on the ratio of the total N and S excretion during administration of diet of adequate caloric value containing 22½ g. protein per day

represented the savings of protein between the 4th and the 9th day corresponding to 3.5 and 3.0 g. per day. The nitrogen retention brought about by TPP during the first 12 days amounted to a total of 3.0 g. corresponding to 18.8 g. protein, with a saving between the 4th and the 9th day of 14.0 g. or 2.3 g. protein per day.

(3) As no food analysis was made, it was not possible to compare the balances of the protein catabolites, so that we had to be content with recording the proportions of the excretions. Fig. 12 shows the N/S ratios. Although this method is less accurate, the impression is gained that every time NAPP or TPP was administered, relatively more sulphur than nitrogen was excreted, while MAD had a similar but less pronounced influence. Twelve to fifteen days after the injections, the initial proportion of the excretions was restored. The significance of this observation will be discussed later.

Fig. 13 Exp. II Effect of anabolic steroids on the N balance during administration of diet of adequate caloric value containing 137 g. protein per day

(4) After the quantity of protein in the diet had been increased from 22½ to 137 g. per day nitrogen equilibrium was achieved during the first period of 3 days (fig. 13). Thereafter the nitrogen balance became very markedly positive, while 18 days after the beginning of the high-protein diet there was again a nitrogen equilibrium. The positive balance was the consequence of the protein losses during the first part of the experiment. In 90 days 393 g. protein were lost, while the body-weight



Fig. 10 – Exp II – Effect of anabolic steroids on the excretion of P during administration of a diet of adequate caloric value containing 22½ g. protein per day

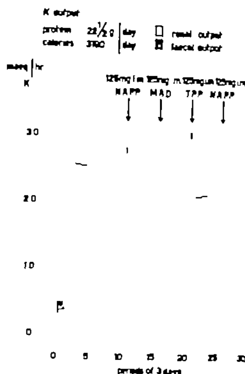


Fig. 11 – Exp II Effect of anabolic steroids on the excretion of K during administration of diet of adequat caloric value containing 22½ g. protein per day

diet as appears from the experiments I IV and IX.

The excretions of phosphorus, sulphur and potassium no longer showed distinct alteration during the last part of the period in which no hormones were administered yet (figs 9 10 and 11).

(2) As a reaction to the administration of NAPP and TPP the nitrogen excretion decreased considerably the protein-saving influence of MAD was found to be less pronounced (fig. 8) The saving effect of NAPP and TPP was exhausted after approximately 12 days. The period between the injections was too short to be able to judge whether the gain of nitrogen was permanent or would be wholly or partially compensated by a greater excretion After the injections, the nitrogen balance was improved, but here also the terminal period was too short to afford certainty concerning the duration of the improvement. It is possible that the presumed coincidental protein-catabolic factor had ceased to exist at the end of the experiments.

Although, therefore, this experiment yields few reliable data concerning the definite saving effect of NAPP MAD and TPP it does give a certain insight into the degree of nitrogen gain during the first 15 days after administration of the steroid. The maximal gain was always observed from the 4th to the 9th day inclusive. The additional retention brought about by NAPP amounted to 5.3 and 3 7 g nitrogen in 12 days, corresponding to 33 1 and 23 0 g. protein Of this, 20.8 and 18 0

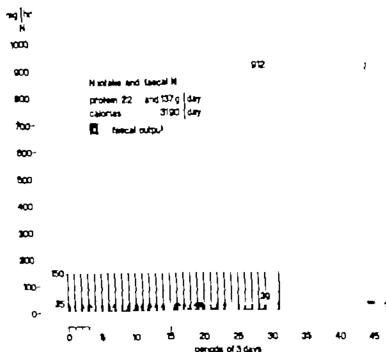


Fig. 15 Exp. II Influence of amount of protein in the diet on the faecal excretion of N

(7) The haemoglobin concentration showed hardly any change during the 2 diets. The serum protein concentration also was hardly influenced, although it appeared that the albumin fraction decreased during the low protein diet, a decrease that was cancelled after the change of diet.

(8) Important alterations of the blood urea concentration were observed only during the first few days after a change of the diet. At the end of the period with the low-protein diet the blood contained 174 mg. urea per litre, 9 days later 505 mg. and at the end of the experiment 494 mg. If we consider only the intake and the excretion of nitrogen, we might calculate a protein synthesis of 105.3 g. for the first 9 days of the high-protein diet. However in this period the blood urea concentration increased by 331 mg. per litre so that the quantity of urea in the body increased by 21.5 g., corresponding to approximately 65 g. protein. The actual synthesis of protein therefore was not 105.3 g. but 40.3 g., i.e. only 4.5 g. per 24 hours.

(9) Fig. 15 shows the average excretion of nitrogen with the faeces as against the intake of nitrogen with the food. With the low-protein diet, 35 mg. nitrogen per hour were excreted with the faeces, corresponding to 5.25 g. protein per day and with the high-protein diet, 39 mg. nitrogen per hour were found in the faeces, corresponding to 5.85 g. protein per 24 hours. After the protein in the diet had been increased by 114½ g. per day the faecal

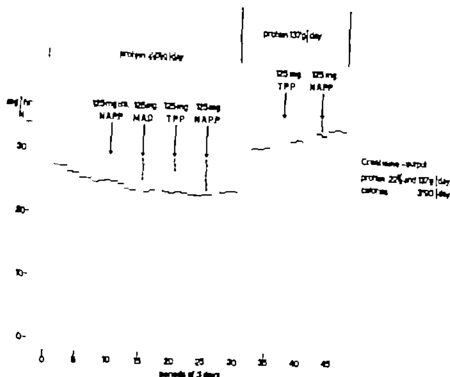


Fig. 14 Ex II – Excretion of creatinine during administration of diets of adequat caloric value containing [illegible] g. and 137 g protein per day respectively

decreased by only 400 g The second diet caused a protein synthesis of 161 g. in 21 days with the body weight increasing 1 9 kg.

The differences in the excretion of the protein catabolites of the various 3-day periods were greater than with the low-protein diet which fact in all probability was caused by the daily oscillations of the quantity of protein in the large portions of protein rich food (meat, cheese).

(5) Intramuscular administration of 125 mg. TPP or 125 mg. NAPP caused a marked nitrogen retention which started sooner and reached a greater d-gree than during administration of the low protein diet. The saving by TPP in 6 days a mounted to 10 0 g. nitrogen correspond ing to 63 0 g. protein or 10 5 g. per day

After administration of NAPP 9.2 g. nitrogen was retained in 6 days, which corresponds to 57 6 g. protein or 9 6 g. per day

(6) Fig. 14 shows the daily excretion of creatinine in the course of the experiment. During the low protein diet a gradual decrease occurred, which after 4 injections appeared to change into a slight increase. The decrease is an indication that muscular tissue is disintegrated No linear proportionality with the quantity of muscular tissue existed however because the de crease of the creatinine excretion amounted to more than 25 %, whereas less than 400 g. protein were lost during this period. The urine immediately after the switch to the high-protein diet, contained considerably more creatinine, presumably as th conse quence of the increased intake of creatinine with the food During the period of the high-protein diet the quantity of creatinine showed a gradual further increase

Fig. 16. Exp. III. ♂ 48. Excretion of protein catabolites during administration of diets containing from 20.4 to 22.5 g. protein, and with caloric value, increasing from 500 to 3500 calories per day.

entific work. Obviously the psychological conditions for the suppression of disagreeable sensations were highly favourable.

With the diets yielding 1000 and 1500 calories, the loss of weight grew less, and no further loss was noted after the caloric value had been increased to 2000. It must be noted in this connection that practically no physical work was done. The weight began to increase again on the diet of 2500 calories. During the last 45 days, when the food contained 3500 calories, the body weight increased 5 kg.

(2) Fig. 17 shows the nitrogen balance. After the previously mentioned rapid decrease of the nitrogen excretion there followed a period of slower improvement of the nitrogen balance. At the end of the period in which the diet yielded 500 calories, some 100 mg. more nitrogen was excreted per hour than was ingested, corresponding to 15.0 g. protein per day. With

nitrogen therefore increased to a degree corresponding to only 0 6 g *protein* This demonstrates clearly how wrong it is to estimate the faecal nitrogen as a percentage of the quantity of protein ingested with the food

III – THE THIRD EXPERIMENT was also carried out in the same healthy man who during this experiment was 48 years old.

The diet always contained approximately 20 g. protein but the caloric value varied During the first 66 days the diet *yielded 500 calories then for 9 days 1000* calories then for 9 days 1500 then for 9 days 2000 then for 9 days 2500 then for 9 days 3000 and *finally for 45 days* 3500 calories per day

The diet consisted for the first 66 days of 50 g. brown bread, 150 g. potatoes, a hen s egg of average size, 20 g. old Edam cheese, 10 g. whipped cream 6 g. sugar 5 g. marmalade 5 g. natural butter and alternately 200 g. chicory 150 g. carrots and 200 ml tomato juice furthermore 1 ml. of the previously described vitamin mixture and 1750 ml. water

The next 500 calories were obtained by adding 30 g sugar 20 g. marmalade and 45 g. butter *All further diets contained* the same foodstuffs, increased by a varying quantity of porridge *which consisted of two* parts sugar two parts natural butter and one part custard powder

The quantity of water was decreased by what had been used in the preparation of the porridge

All foodstuffs have been subjected to chemical analysis, at least twice, with determination of the quantities of nitrogen sulphur phosphorus, potassium and calcium In the preparation of the food, a quantity of sodium chloride was used that was *approximately* the same each day The first diet contained 20 4 g. protein and each subsequent 500 calories corresponded to 0.37 g. protein per day additionally so that the last diet which yielded 3500 calories, contained 22.5 g. protein

Fifteen and forty-five days after the beginning of the experiment 50 mg. NAPP was injected intramuscularly and during the last period, when the diet yielded 3500 calories 20 g. sodium chloride extra were *given per day for 3 days Also 27 days* before the end of the experiment 25 mg. nor androstenolone decanoate (NAD) were injected and 6 days before the end 30 mg. *ethylnor* testosterone *(ANT)* were given orally 3 times at 8-hour intervals.

The urine was practically always collected in 72 hour specimens. In a few instances, the determinations were carried out per 24 hours. At suitable moments, the urea concentration, the proteinpattern and the haemoglobin value of the blood were determined.

This test was carried out in the first place in order to determine what protein-saving value is to be ascribed to the caloric value of the diet As far as possible we always imitated the conditions *prevailing* in patients with severely reduced renal function *who had had a low-protein diet* for a long time

The data are set forth in table III and *shown graphically in fig. 16*

(1) During the first period of 66 days on only 500 calories per day the body weight decreased by *18 kg The subject* experienced *relatively little discomfort from this malnutrition The sensations of hunger did not prevent him from doing sci*

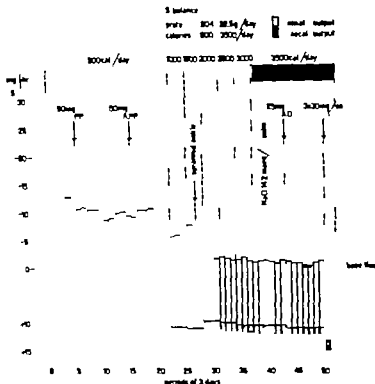


Fig. 18. Exp. III. Effect of gradual increase in the caloric intake on the S balance during administration of diets containing from 20.4 to 22.5 g. protein per day

lesions may cause an enhanced protein catabolism, even when the diet is very poor in protein over a long period.

(4) The sulphur phosphorus and potassium balances (figs. 18, 19 and 20) also became less negative when the caloric value of the diet was increased. Those deviations of this rule that have been observed are attributable to the influence of anabolic steroids, extra salt or the trauma to the ankle.

(5) It appears from fig. 17 that the protein-saving effect of NAPP was slight and that its influence was probably smaller the longer the calorically insufficient diet had been given.

After both NAPP injections it appeared that here, also the excretion of sulphur was relatively higher than the nitrogen excretion (fig. 18), while the excretion of phosphorus decreased relatively more distinctly than that of nitrogen (fig. 19). The potassium balance, owing to a greater irregularity is not convincing (fig. 20).

(6) Administration of 20 g. sodium chloride per day for 3 days during the diet of 3500 calories which already contained a normal quantity of salt caused no changes of the excretion of nitrogen and sulphur whereas the quantities of phosphorus and potassium excreted showed a significant increase (figs. 17 18, 19 20).

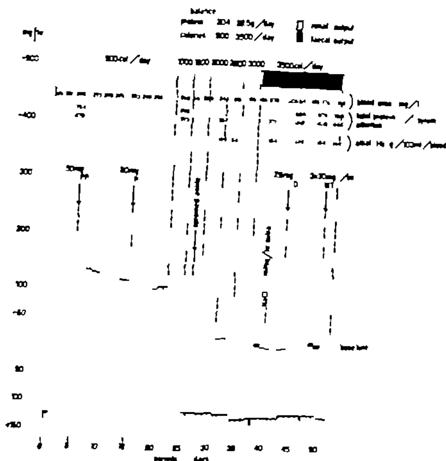


Fig. 17 – Exp III – Effect of gradual increase in the caloric intake on the N balance during administration of diets containing from 20.4 to 22.5 g. protein per day

the 1000-calorie diet, the daily protein loss amounted to approximately 10.5 g. with the 2000-calorie diet, to 3 5 g. and with the 2500-calorie diet to 0 9 g., whereas with the 3000-calorie diet the nitrogen balance had become just positive (0 3 g. protein) With the 3500-calorie diet the positivity had become more pronounced and approximately 1 g. protein per day was ingested above the amount excreted.

As a rule the influence of the changes of the blood urea concentration on the nitrogen balance was negligible

We have found, consequently that with a diet containing 20.4 to 22.5 g. protein

a) *the nitrogen balance became less negative the longer a calorically insufficient diet was continued*

b) the nitrogen balance became less negative after *the caloric value of the diet* was increased

c) increase of the caloric value beyond a value with which the body gains weight further stimulated the protein synthesis.

(3) After the subject had been on the 1500-calorie diet for 2 days, he sprained his left ankle, with development of a small haematoma. This small accident caused an increased excretion of nitrogen so that the influence of the diet on the *metabolism of protein could* not be evaluated This observation indicates that slight

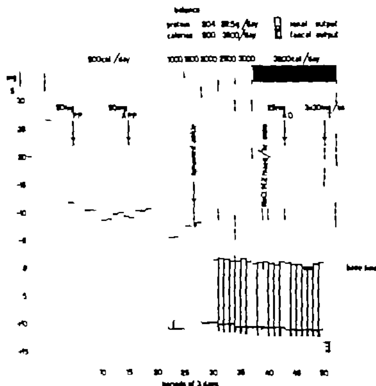


Fig. 18 Exp. III Effect of gradual increase in the caloric intake on the S balance during administration of diets containing from 20.4 to 22.5 g. protein per day

lesions may cause an enhanced protein catabolism, even when the diet is very poor in protein over a long period.

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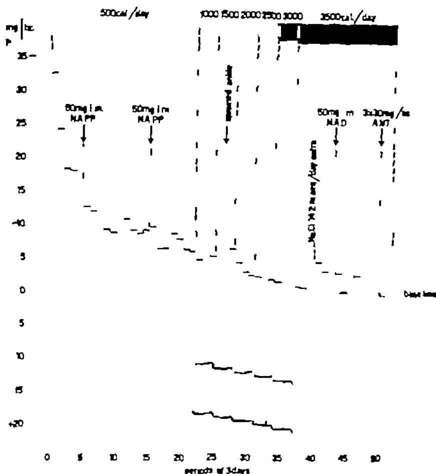


Fig. 19 – Exp III – Effect of a gradual increase in the caloric intake on the P balance during administration of diets containing from 20.4 to 22.5 g. protein per day

(7) *Towards the end of the experiment* first nor-androstenolone decanoate (NAD) and then ethylnor testosterone (ANT) were administered. In other words, these substances were tested for their protein-saving effect after a considerable quantity of body protein had already been lost. After intra muscular administration of 25 mg. NAD *no distinct nitrogen saving was observed* and the excretions of sulphur phosphorus, and potassium were not significantly influenced, either Ethylnor testosterone was administered orally 3 times in a dose of *30 mg. at 8-hour intervals. It brought* about no significant protein-saving effect. *It can be seen in fig. 17 that more nitrogen* was excreted during the first 3 days, and afterwards a little less. A striking fact was, however that the excretion of sulphur increased relatively more (fig. 18). The specimens of urine concerned were ex *amined every 24 hours. From* table III it appears that the additional sulphur ex cretion could be observed only during the first 2 days after the administration of ANT In the graph (fig. 18) the fact is

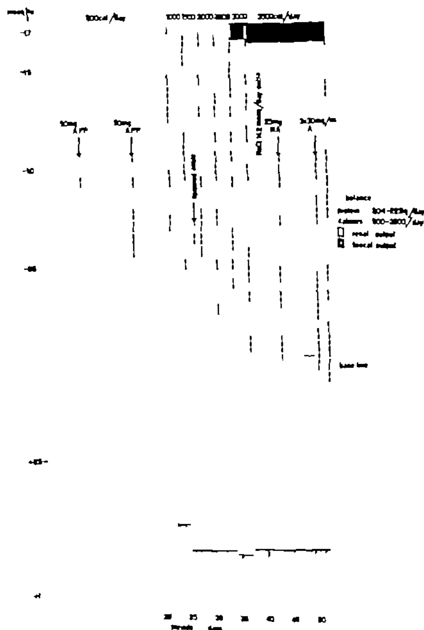


Fig 20 Exp III Effect of gradual increase in the caloric intake on the K balance during administration of diets containing from 20.4 to 22.5 g. protein per day

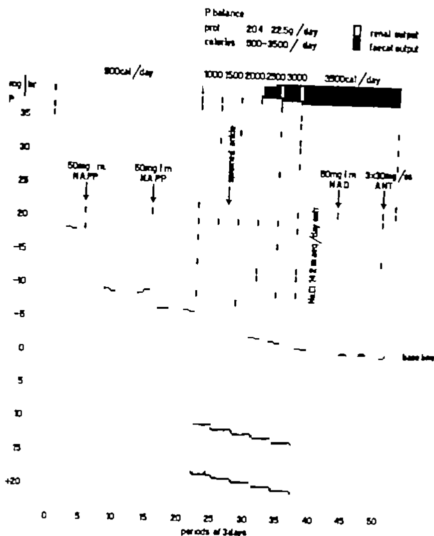


Fig. 19 – Exp. III Effect of gradual increase in the caloric intake on the P balance during administration of diets containing from 20.4 to 22.5 g. protein per day

(7) Towards the end of the experiment first nor-androstenolone decanoate (NAD) and then ethylnor testosterone (ANT) were administered In other words these substances were tested for their protein saving effect after a considerable quantity of body protein had already been lost. After intra muscular administration of 25 mg NAD no distinct nitrogen saving was observed, and the excretions of sulphur phosphorus, and potassium were not significantly influenced, either Ethylnor testosterone was administered orally 3 times in a dose of 30 mg. at 8-hour intervals. It brought about no significant protein saving effect. It can be seen in fig. 17 that more nitrogen was excreted during the first 3 days and afterwards a little less. A striking fact was, however that the excretion of sulphur increased relatively more (fig. 18) The specimens of urine concerned were ex amined every 24 hours. From table III it appears that the additional sulphur ex cretion could be observed only during the first 2 days after the administration of ANT In the graph (fig. 18) the fact is

date	caloric value of the diet per 24 hours	g fat in the diet per 24 hours	total fat in the faeces in g per 24 hours
29-9-'58 to 3-12 58	500	22	1 13
4-12 58 to 12 12 '58	1000	60	2.23
13-12 58 to 8-1 59	1500 to 2500	93 to 159	3 14
9-1 '59 to 17 1 59	3000	192	3.84
18-1 59 to 3-3 59	3500	225	4.08

The difference of the loss of calories with the faeces in the periods with 500-calorie diet and with 3500-calorie diet, respectively consequently amounted to only 27 calories per 24 hours, approximately

IV THE FOURTH EXPERIMENT was carried out in a healthy woman who received a daily diet which contained 22 g protein and yielded 2250 calories. The food consisted of the same components as in the previous experiment the custard porridge yielded 1250 calories per day Eighteen days after the beginning 25 mg. NAPP were injected intramuscularly and 27 days later 25 mg. NAD

After 81 days, this diet was replaced by one that consisted exclusively of 100 g. sugar 2800 ml. tap water 9.2 g. NaCl, 4.8 g. $KHCO_3$ and 1 ml. of the vitamin mixture described previously This diet was given for 6 days, after which, for 12 days the quantity of sugar was raised to 300 g. per day After this last-mentioned diet had been continued for 6 days, ANT was administered, in a dose of 3×30 mg at 8-hour intervals. In the course of the experiment the subject performed fairly light household duties.

The data are listed in table IV and shown graphically in fig. 22.

(1) In the course of the first 81 days the body-weight decreased from 56.2 to 53 kg.

It appears from fig. 23 that the nitrogen balance was continuously negative. Toward the end of this period the catabolism of protein exceeded the synthesis by some 7 g. per day The sulphur phosphorus and potassium balances show a similar appearance (figs. 24 25 and 26).

(2) After administration of 25 mg. NAPP there were a dubious nitrogen saving, a relatively higher sulphur excretion and a slightly more pronounced retention of phosphorus and potassium. After administration of NAD no nitrogen saving could be observed. The three-day period which began 3 days after the injection was characterized by an increased excretion of sulphur (fig. 24).

(3) When the diet was changed to 100 g. sugar per day the quantity of nitrogen in

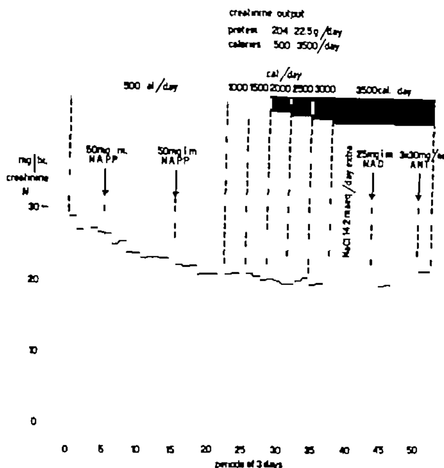


Fig. 21 – Exp III – Creatinine excretion during administration of diets containing from 20 4 t 22.5 g. protein, and with a caloric value from 500 to 3500 calories per day

taken into account that the tablets in addition to 10 mg ANT contained 85 mg. sodium sulphate It appears from fig. 19 *that ANT caused a decrease of the* phosphorus excretion which persisted for 2 × 24 hours (table III)

(8) During the periods on the low calorie diets the excretion of creatinine gradually decreased which may indicate a decrease of the total muscle mass Here, also however the decrease of the creatinine excretion was more than could be explained by the loss of muscle. This observation will be discussed later After the caloric intake had been increased, the creatinine excretion remained approximately stable (fig. 21).

(9) During the first 132 days a total of 296 g. nitrogen was excreted in excess of the intake which corresponds to 1850 g. *protein If we assume that the quantity of* circulating blood did not change, approximately 56 g. serum protein was lost in this period and *100 g. haemoglobin*, while the total quantity of urea in the body decreased by 11 4 g., corresponding to some *34 g. protein (table III)*

(10) In order to determine how many calories were lost through faecal excretion the *faeces were examined* for fat (by the method of VAN DE KAMER TEN BOKKEL HUININK and WEYERS GORTER and *DE GRAAFF 1956*).

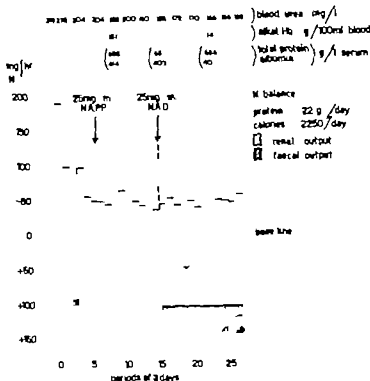


Fig 23 Exp IV Effect of anabolic steroids on the N balance during administration of diet of almost adequate caloric value containing 22 g. protein per day

In comparing the nitrogen balances during the feeding with 100 and with 300 g. sugar the first 2 days after a change of diet have not been considered in accordance with the advice of REIFENSTEIN, ALBRIGHT and WELLS (1945)

It appears from fig. 27 that the nitrogen excretion during the diet of 100 g. sugar amounted to 146 mg. per hour on the average, whereas after the quantity of sugar had been increased to 300 g., an average of 120 mg. nitrogen was excreted per hour It can be calculated, therefore, that first 21 9 and then 18.0 g. protein per day was lost. We find therefore that under conditions in which presumably the body handles its protein economically an increase of the quantity of sugar from 100 to 300 g. causes a protein saving of only 3.9 g. per 24 hours. When the quantity of variable nitrogen in the urine during both diets is calculated, we find that 14 1 and 10.7 g. protein respectively were lost per day The gain obtained by increasing the quantity of sugar in the diet therefore amounted to 3 4 g. protein, or 24 / of the catabolism during the diet of 100 g. sugar per day The question whether this quantitatively slight influence of 200 g. sugar on the protein metabolism is actually correlated with the long duration of the previous, markedly low-protein diet will be answered by a subsequent experiment.

(4) After administration of ANT during

Fig. 22 – Exp IV ♀45 Excretion of protein catabolites during administration of diet of almost adequat caloric value containing 22 g. protein per day followed by exclusive sugar diet in various quantities.

the body had decreased by approximately 110 g. corresponding to 690 g. protein Before determining the loss of body protein over this period we must deduct some 17 g. from this quantity because of the decrease of the quantity of urea in the body while protein had also been lost during 4 menstrual periods. Owing to this loss of protein the condition of the body at the beginning of the sugar diet was probably one in which the body was able to economize in the metabolism of protein

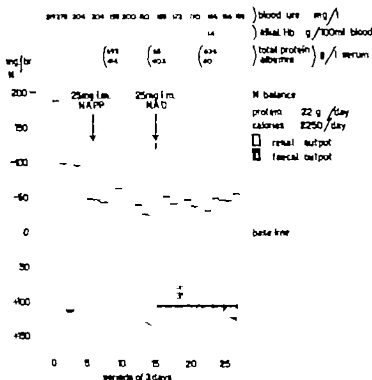


Fig. 23 Exp IV Effect of anabolic steroids on the N balance during administration of diet of almost adequate caloric value containing 22 g. protein per day

In comparing the nitrogen balances during the feeding with 100 and with 300 g. sugar the first 2 days after a change of diet have not been considered in accordance with the advice of REIFENSTEIN, ALBRIGHT and WELLS (1945).

It appears from fig. 27 that the nitrogen excretion during the diet of 100 g. sugar amounted to 146 mg. per hour on the average whereas after the quantity of sugar had been increased to 300 g., an average of 120 mg. nitrogen was excreted per hour. It can be calculated, therefore, that first 21.9 and then 18.0 g. protein per day was lost. We find therefore that under conditions in which presumably the body handles its protein economically an increase of the quantity of sugar from 100 to 300 g. causes a protein saving of only 3.9 g. per 24 hours. When the quantity of variable nitrogen in the urine during both diets is calculated, we find that 14.1 and 10.7 g. protein respectively were lost per day. The gain obtained by increasing the quantity of sugar in the diet therefore amounted to 3.4 g. protein or 24 % of the catabolism during the diet of 100 g. sugar per day. The question whether this quantitatively slight influence of 200 g. sugar on the protein metabolism is actually correlated with the long duration of the previous, markedly low-protein diet will be answered by a subsequent experiment.

(4) *After administration of ANT during*

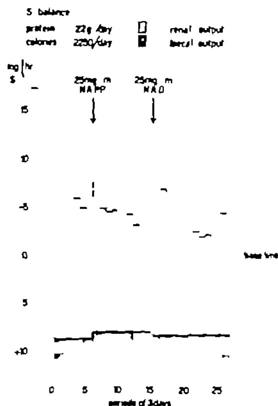


Fig. 24 – Exp IV – Effect of anabolic steroids on the S balance during administration of diet of almost adequat caloric value containing 22 g protein per day

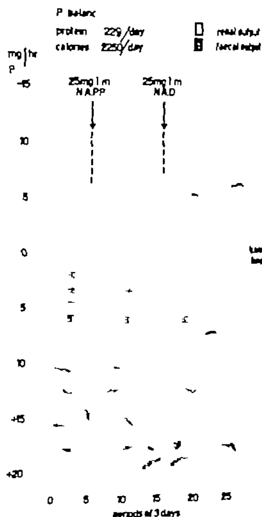


Fig. 25 Exp IV Effect of anabolic steroids on the P balance during administration of a diet of almost adequat value containing 22 g protein per day

the feeding with 300 g. sugar per day the following findings were seen

a) no change of nitrogen balance (fig. 27)

b) a pronounced increase of the excretion of sulphur which lasted some 24 hours. In fig. 28 the sulphur present in the tablets has been taken into account

c) retention of phosphorus, which lasted 2 days (fig. 29)

d) possible retention of potassium. However the irregularity of the excretion curve renders this observation problematic (fig. 30)

The changes in the balances under the influence of ANT are presented in a different manner in figs. 31 and 32. Here we find that in the absence of any alteration of the nitrogen excretion the sulphur excretion has increased considerably and the phosphorus balance showed a dubious improvement. The possible explanation of these observations will be discussed in a separate chapter

(5) During the feeding with 100 g. sugar the urine contained no acetone and no glucose could be demonstrated in the urine when the diet consisted of 300 g. sugar

V – THE FIFTH EXPERIMENT was carried out in 2 healthy men A and B both of them

19 years old, and weighing 76.6 and 65.0 kg. respectively

The diet consisted during the first 5 days of 440 g. brown bread, a hen's egg of average size, 100 g old Edam cheese 10 g. whipped cream, *16 g. sugar 60 g.* marmalade, 20 g. powdered milk, 200 ml. *tomato juice* and 145 g. natural butter for the heavier man per 24 hours and 85 g. per 24 hours for the other

Furthermore both men received 1 ml. per day of the vitamin mixture previously described and 1750 ml. water This complete diet contained 80 g. protein and yielded 38.9 calories per kg. body-weight. During this period the men performed normal light work.

Immediately subsequent to this period there followed a period of bed rest, which also lasted 5 days, and during which, in addition to water 9.2 g. NaCl 4 8 g. $KHCO_3$ and vitamins, only sugar was consumed in quantities of 100 and 400 g. per day respectively in 4 equal portions.

During the next 5 days no diet was prescribed, and for the subsequent 6 days the diet containing 80 g. protein was given *again* Thereafter bed *rest* was *imposed* again duri g which the heavier man, who had first consumed 100 g. sugar per 24 hours, now obtained 400 g., whereas the other consumed 100 instead of 400 g. per day

In this way therefore t was possible to compare the excretion of protein catabolites with ugar diets of 100 and 400 g. I *contradistinction* to the previous experiment, in which this comparison was made after a protracted period of low-protein diet, the present experiment concerns optimally fed persons, who suddenly ate sugar exclusively Whereas in the previ-

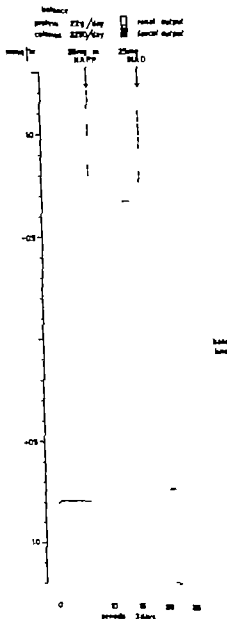


Fig. 26 Exr IV Effect of anabolic steroids on the K balance during administration of diet of almost adequate caloric value containing 22 g. protein per day

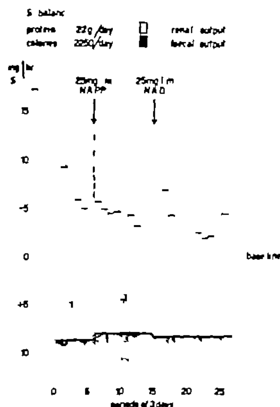


Fig. 24 – Exp IV – Effect of anabolic steroids on the S balance during administration of diet of almost adequate caloric value containing 22 g. protein per day

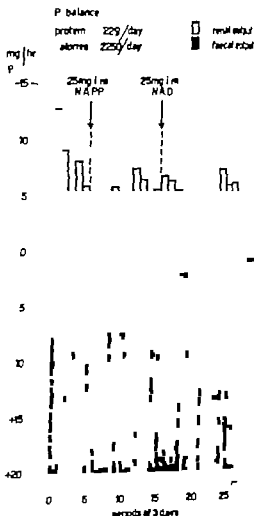


Fig. 25 Exp IV Effect of anabolic steroids on the P balance during administration of a diet of almost deqaate value containing 22 g. protein per day

the feeding with 300 g. sugar per day the following findings were seen

a) no change of nitrogen balance (fig. 27)

b) a pronounced increase of the excretion of sulphur which lasted some 24 hours In fig. 28 the sulphur present in the tablets has been taken into account

c) retention of phosphorus which lasted 2 days (fig. 29)

d) possible retention of potassium However the irregularity of the excretion curve renders this observation problematic (fig 30).

The changes in the balances under the influence of ANT are presented in a different manner in figs. 31 and 32. Here, we find that in the absence of any alteration of the nitrogen excretion the sulphur excretion has increased considerably and the phosphorus balance showed a dubious improvement. The possible explanation of these observations will be discussed in a separate chapter

(5) During the feeding with 100 g sugar the urine contained no acetone and no glucose could be demonstrated in the urine when the diet consisted of 300 g. sugar

V – THE FIFTH EXPERIMENT was carried out in 2 healthy men, A and B, both of them

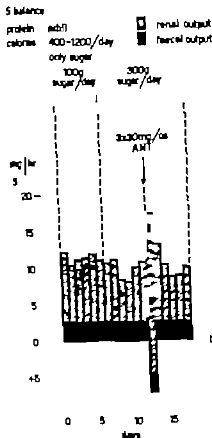


Fig. 28 Exp. IV Effect of increasing the amount of sugar on the S balance during administration of sugar only *following long-term administration of diet poor in protein.*



Fig. 29 Exp. IV Effect of increasing the amount of sugar on the P balance during administration of sugar only *following long-term administration of diet poor in protein*

creased when the protein-containing food was replaced by 400 g. sugar but increased when 100 g. sugar were given. The figs. 36 and 38, in which the alterations of the blood urea concentration have been taken into account, therefore give a more reliable impression of the degree of protein catabolism during the various diets.

It appears from these figures that in spite of the striving for uniformity there are still differences between the excretions during the first and the second periods of standardized, protein-containing food. In both subjects the first periods were characterized by a negative nitrogen balance, whereas during the second experiment nitrogen equilibrium was obtained in both cases. The question may be raised whether the negative nitrogen balance during the first control period does not necessitate a correction of the observed effect of the sugar meals on the protein catabolism. With application of the maximal correction the calculated nitrogen gain, due

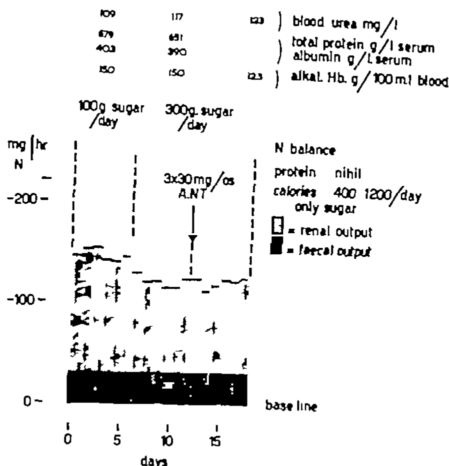


Fig. 27 – Exp IV – Effect of increasing the amount of sugar on the N balance during *administration of sugar only following long-term administration of diet poor in protein*

ous experiment the nutritional condition resembled that of uraemic patients who had been kept on a low protein diet for a long time, the present experiment can be compared to the sudden development of anuria in well fed, otherwise healthy persons.

All *nutrients have been analysed, with* determination of the levels of nitrogen sulphur phosphorus, potassium and calcium. *During the period of standardized* feeding 24-hours specimens of urine were examined One specimen of both test persons *was lost. The faeces were collected* per period but in this case also the accuracy was not great because of the lack of a good labelling method. The urea concentration of the blood was determined regularly

The data have been listed in table V (A and B) and are shown graphically in figs. 33 and 34

(1) The nitrogen balances, calculated *from the uptake with the food and ex* cretion with urine and faeces are shown in figs. 35 and 37 As remarked previously *such a balance does not give an accurate* impression of the protein metabolism, when the blood urea concentration under goes important changes during the ex periment. It appears from the table that the urea concentration in both cases de

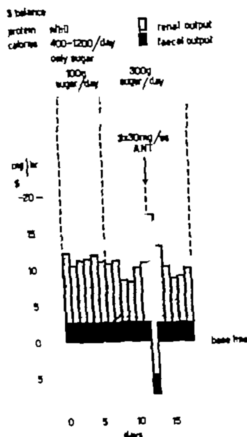


Fig. 28 Exp. IV Effect of increasing the amount of sugar on the S balance during administration of sugar only *following long-term administration of diet poor in protein.*

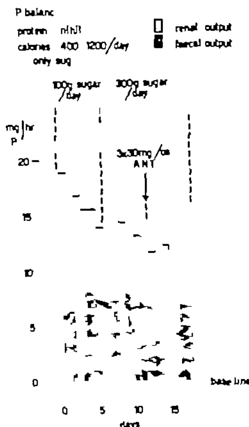


Fig. 29 Exp. IV Effect of increasing the amount of sugar on the P balance during administration of sugar only *following long-term administration of diet poor in protein.*

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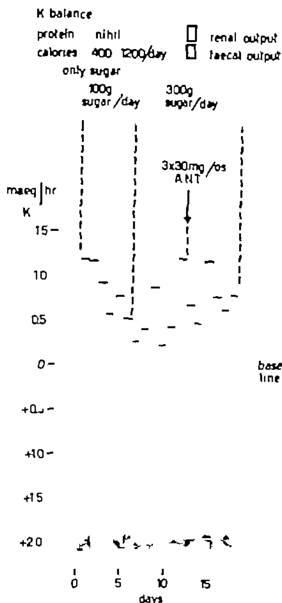


Fig. 30 Exp IV Effect of increasing the amount of sugar on the K balance during administration of sugar only *following long-term administration of diet poor in protein*

to the larger sugar meals becomes smaller in one of the cases, and about as much greater in the other case than when no correction was applied

Figs. 39 and 40 give a picture of the differences in cumulative protein catabolism during feeding with 100 and 400 g. sugar The excretions of nitrogen with the urine *and faeces have been taken into account,* as have the daily variations of the blood urea level Fig. 39 shows that after 4 days there was a difference in protein catabolism of 117 5 g. in favour of the regime with 400 g. sugar In the other experiment, also, a distinct decrease of protein catabolism under the influence *of the increase of* caloric intake was observed (fig. 40). Here the difference after four days amounted to 47 9 g protein From these cumulative balances it appears that after 4 (5) days there was as yet no tendency to decrease the difference between the nitrogen excretions under the influence of 100 and of 400 g. sugar

The figs. 41 and 42 give an impression of the calculated increase of the blood urea concentration, cumulative during the first 4 days, if an anuria would have been present The differences between the diets are in both cases considerable For the assessment of the compared diets in a case of acute anuria figs. 41 and 42 can best be used because here the degree of accumulation of protein catabolites is of great importance, whereas the changes of the nitrogen balance in view of the brief duration of the treatment, are not significant. A comparison of the results of experiments IV and V will be made later A number of the data are listed in table XIX

The results obtained in the fifth experiment *show a considerable disparity* with the findings reported by GAMBLE (1946). For purposes of comparison with figures 39 and 40 in figure 43 we present a copy of a curve described by Gamble By giving a fasting man 100 g. glucose per 24 hours a significant protein saving was obtained but a larger quantity of glucose caused practically no extra decrease of the protein

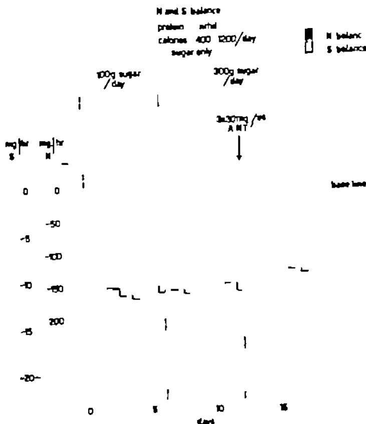


Fig. 31 Exp IV Effect of ethylnor-testosterone (Nilevar ANT) on the N and S balances during administration of diet consisting exclusively of 300 g sugar following long-term administration of diet poor in protein

catabolism. However alterations of the blood urea level have not been taken into account, and after a 6-day period of fasting it is quite possible that a large part of the depot protein had already been lost, causing a similarity with the 4th experiment in which a low-protein diet had been administered for a long time. It is incorrect to use Gamble observations in the assessment of the composition of diets of patients with decreased renal function, as MERRILL (1955 1960) has done.

(2) The excretions of the remaining protein metabolites sulphur phosphorus and potassium were also distinctly smaller during the calorically richer sugar diet, as appears from figs. 33 and 34 Because no determinations have been made of the quantities of these substances in the blood, however we do not obtain an accurate impression of the degree of protein saving under the influence of different quantities of sugar

The differences were considerable, however as appears from the urinary excretions of potassium. One of the men excreted 23 m.eq. potassium in 4 days during the diet of 100 g. sugar whereas

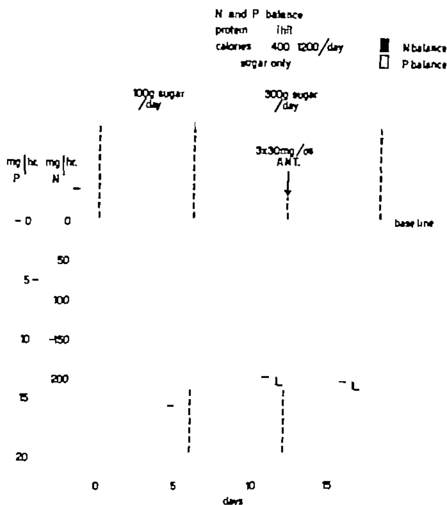


Fig. 32 – Exp IV – Effect of ANT on the N and P balances during administration of diet consisting exclusively of 300 g. sugar following long-term administration of diet poor in protein.

over the same length of time during the diet of 400 g. of sugar 170 m.eq were found in the urine. For the other man these figures were 236 and 169 m.eq respectively In both cases therefore there was an extra loss of potassium of more than 60 m.eq during the diet with the lowest caloric value, which corresponds to 15 m.eq potassium per 24 hours.

In the following experiments the conditions have been slightly changed and in some respects more strictly standardized. It has been demonstrated by KASSENAAR (1952), that in the determination of the anabolic effect of various steroids, experiments, in which only the weight or the composition of an animal organ is determined are not conclusive He arrives at the conclusion that the only correct measure of the protein anabolic activity of a substance is its effect on the protein balance Moreover it has been known for a long time and has been stressed once more by Munro (1951), that balances may lose a considerable part of their significance when the duration of the experiment has been too short. Therefore we have attempted by rigid standardization

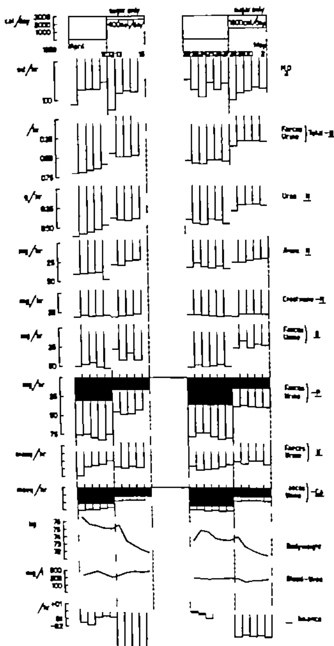


Fig 33 Exp V A ♂ 19 Influence of the quantity of sugar on the excretion of protein metabolites, during the administration of sugar only *following administration of an optimal diet.*

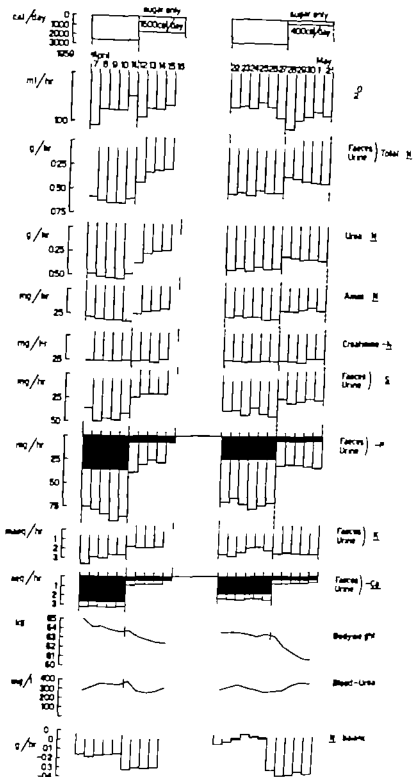


Fig. 34 Exp V B ♂ 19 Influence of the quantity of sugar on the excretion of protein metabolites, during the administration of sugar only *following administration of an optimal diet.*

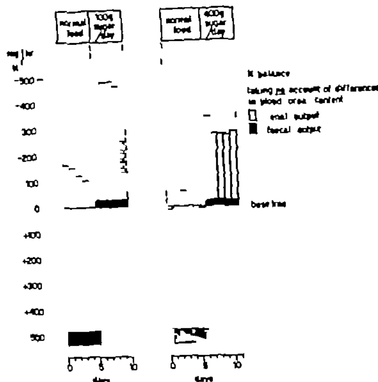


Fig. 35 Exp. V A Influence of the quantity of sugar on the N balance during the administration of sugar only following administration of an optimal diet No corrections for differences in blood urea level.

to shorten the duration of the experiments without losing reliability and accuracy of the conclusions to be drawn from the findings.

The experiments have been carried out in students who for this purpose stayed at the author's home. The food was administered at 3-hours intervals, mostly in 8 equal portions. During the whole duration of the experiment the subjects remained in bed with the exception of 8 occasions of a brief period during which the urine was oided after which the test person carried t to a refrigerator In order to diminish atrophy due to inactivity they made as many muscular movements as possible during this short walk (approximately 50 m). A warm shower was taken once per day always at the same time. Further the students left their bed, naturally at ir regular times, for defaecation.

As it was the purpose of the experiment to determine whether the separate administration of the different nutrients exerted influence on the excretion of protein catabolites, foodstuff of constant composition had to be selected which contained exclusively one of the three elements of the food. For the carbohydrate beet sugar was selected and for the fat natural butter In choosing the protein it was naturally necessary to consider the composition and di-

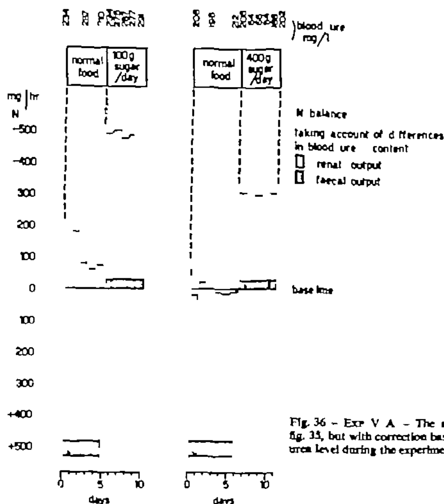


Fig. 36 – Exp V A – The same N balance as in fig. 35, but with correction based on changes in blood urea level during the experiment.

gestibility For practical reasons, milk protein has been selected a substance that, in the previously mentioned table of MITCHELL and HAMILTON (in DOYER, 1951) was ranked as the highest but one

It is only the protein from hen s eggs (a mixture of white and yolk) which is given a higher biological value in this table The milk protein was furnished by the firm of Blomberg in The Hague It is a mixture of the 3 principal milk proteins, casein lact albumin and betalactoglobulin These proteins are obtained from fresh milk by a process of simultaneous precipi tation The nature of this process is a trade secret The manufacturer uses this protein, after addition of taste correctives, to make the preparation proteinol.

All experiments have been carried out with only two homogenic supplies of milk protein, that was consumed without taste correctives. The quantities of amino-acids in this milk protein as stated by the manufacturer are in complete agreement with the composition of casein (DOYER, 1951), with the exception that for methionine 4 1 g. per 16 g. nitrogen is given, whereas Doyer mentions 3.5 g. When we compare the quantities of the amino-acids in 70 g. of the milk protein with the minimal requirements (ROSE, 1949) of an adult person we find that a few of the essential amino-acids are present in insufficient quantities

Amino-acids	*Quantity in 20 g. milk protein (grammes)*	*Minimal requirement adult (grammes)*
Arginine	0.8	0
Lysine	1.6	0.8
Histidine	0.5	0
Phenylalanine	1.0	1.1
Tyrosine	1.4	0
Tryptophan	0.3	0.25
Proline	1.6	0.25
Cystine	0.06	0
Methionine	0.8	1.1
Leucine	2.0	1.1
Isoleucine	1.3	0.7
Valine	1.3	0.8
Threonine	0.8	0.5
Serine	1.5	0
Alanine	0.6	0
Glycine	0.1	0
Glutamic acid	4.8	0
Asparaginic acid	1.3	0

On the basis of this table it can be predicted that optimal conditions will not be obtained when the diet contains 20 g. milk protein as the only source of nitrogen. OSBORNE and MENDEL (in SHERMAN, 1952) observed that rats showed optimal growth on calorically adequate diets, when the source of nitrogen was casein in a proportion of 18 of the dry weight of the food, whereas they exhibited considerably retarded growth when the diets contained only 9 casein. This retardation of growth could be prevented by adding cystine or methionine to the food. FELIX (in THANNHAUSER, 1957) states that the body has a tendency to retain animal protein to a larger degree than vegetable protein, but that casein in this respect compares badly with other animal proteins. These data constitute a further indication that low-protein diets with milk protein as the only source of nitrogen will result in a relatively unfavourable nitrogen balance. However in practice it proved not possible to find a better and more feasible method for obtaining a pure protein with a high biological value.

The milk protein proved to contain neither carbohydrates nor fat. Per 100 g. it was found to contain 2 m.eq. potassium and 25.8 m.eq. sodium. The first quantity of the preparation delivered contained per gramme 131.2 mg. nitrogen, 6.8 mg. sulphur and 10.08 mg. phosphorus. For the second batch these figures were 117.6, 5.76 and 9.96, respectively. The fairly high percentage of phosphorus is an indication that the proteinol contains conjugated proteins, in which phosphoric acid is esterified with one of the amino-acids of the protein molecule (caseinogen).

Furthermore, the subjects every 3 hours were given 10 ml. of a beverage which contained 1.15 g. NaCl, and 0.6 g. $KHCO_3$ per 10 ml., and finally they received also 8 times per 24 hours, ½ ml. of the previ-

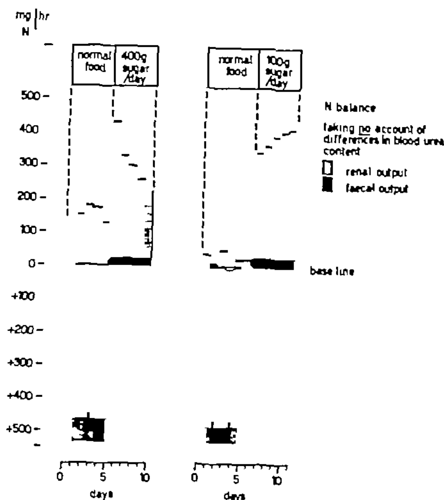


Fig. 37 Exp V B – Influence of the quantity of sugar on the N balance during the dministration of sugar only *follo ing administration of an optimal diet* N corrections for differences in blood urea level.

ously described aqueous multivitamin mixture

It was hoped that this standardization would result in a certain pattern of ex cretion which might serve to reveal small variations that might go unnoticed under the usual experimental conditions. This so-called rhythmic diet has proved to be of great value for the study of the excretion of minerals, in experiments in the clinic of Prof Borst.

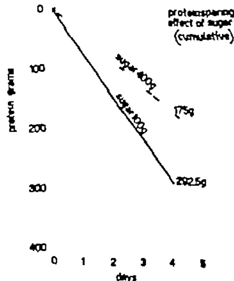
However the daily oscillations in the excretion of protein catabolites were still not insignificant in spite of the standard ization, so that in general there was little use in examining the urine in many portions per 24 hours.

In the following experiments the subjects received the above mentioned rhythmic diet.

VI – THE SIXTH EXPERIMENT was carried out in a healthy man aged 21 years, who 8 times per day ate a mixture of 12 g. milk protein (first batch), 28 g. sugar and 12 g. natural butter with a total of 2000 calories. The findings are listed in table VI and shown graphically in fig 44

Fig. 38 Exp. V B The same N balance as in fig. 37 but with correction based on changes in blood urea level during the experiment.

Fig. 39 Exp. V A Cumulative negative protein balance during the administration of diets, consisting of sugar only *following administration of an normal diet*. Protein-sparing effect of larger amount of sugar



(1) Four days after the beginning of the experiment the subject developed fever the cause of which could not be detected. This fever was associated with a retention of phosphorus (fig. 44).

(2) One week later the experiment could be continued, nd 5 days after that time the milk protein was replaced by a mixture of milk protein and methionine which contained per gramme 125.5 mg. nitrogen, 12.3 mg. sulphur and 9.58 mg. phosphorus. After 4 days this mixture was again replaced by the original food, which was then continued for 3 more days.

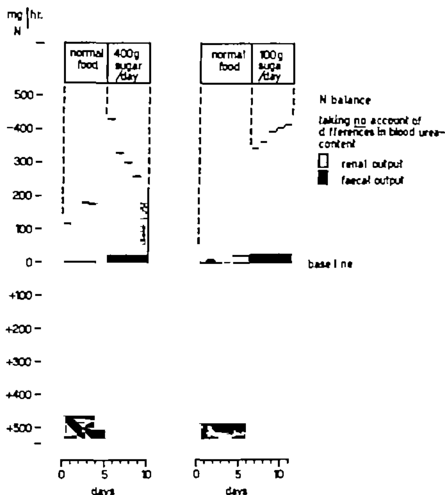


Fig. 37 – Exp V B – Influence of the quantity of sugar on the N balance during the administration of sugar only *following administration of an optimal diet* N corrections for differences in blood urea level.

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Fig. 45 shows the nitrogen balance, and the intake and excretion of sulphur with the urine is shown graphically in fig. 46.

Determination of sulphur and phosphorus in the faeces was not possible in this experiment because the portions were marked with carmine red which had a disturbing influence on the colorimetrical and nephelometric determinations. These figures show that not only a large quantity of sulphur was excreted after methionine had been added to the diet, but that this addition was also followed by a more markedly negative nitrogen balance. This is probably attributable to the fact, which has been observed more than once, that an excess of an amino-acid in the food may result in a less complete utilization of other amino-acids. Examples of this phenomenon, especially also where methionine was concerned, have been given in the review of the literature.

(3) The excretion of ammonia was found to increase during sleep. The respiration is shallower and less frequent, so that less carbon dioxide is exhaled. This leads to a relative acidosis, which is normalized by the excretion of ammonium salts. It was found that this is a very rapid reaction of the kidney. When the subject went to sleep at 11 p.m. instead of at midnight, the fact was noticeable from the excretion of ammonia with the urine voided at midnight (fig. 47). PETERS and VAN SLYKE (1946) has e pointed out that as a rule acidosis is combated first with the aid of the buffers present in the blood, and that the excretion of ammonium salts only follows later on. The combating of the acidosis during sleep constitutes an exception to this rule.

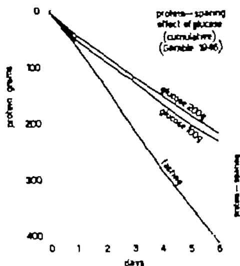


Fig. 43. Exp. V. Protein-sparing effect of diets consisting exclusively of various amounts of sugar following fasting for 6 days (GAMBLE, 1946).

VII – THE SEVENTH EXPERIMENT was carried out in a healthy man aged 23 years who over a period of 19 days was given a fat free diet consisting of 80 g. of the previously described mixture of milk protein and methionine, plus 456 g. beet sugar together yielding 2144 calories per day. Water, NaCl, $KHCO_3$ and vitamins were administered in the dosages mentioned above.

During the first 4 days the 8 portions were equal. Thereafter for 6 days the sugar was given in 4 equal portions, at midnight and at 3 6 and 9 a.m., and the protein, also in 4 equal portions at noon, 3, 6 and 9 p.m. After these 6 days there followed a period as at the beginning of the experiment. On the 16th day the influence of 100 mg. animal growth hormone, injected intramuscularly was examined.

The findings are listed *in table VII* and have been graphically recorded in fig. 48.

(1) The nitrogen balance was clearly negative (fig. 49). On the most favour

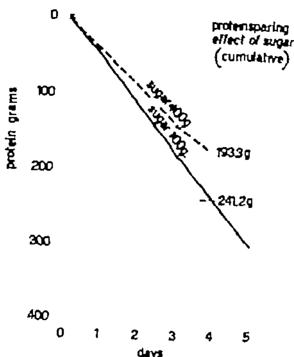


Fig. 40 – Exp V B – Cumulative negative protein balance during the administration of diets, consisting of sugar only *following administration of an optimal diet*. Protein-sparing effect of a larger amount of sugar

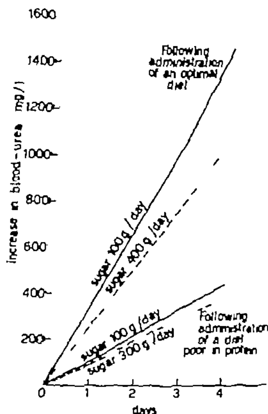


Fig. 42 Exp V B Calculated rise in blood urea in case of assumed acute anuria treated with diets consisting exclusively of respectively 100 and 400 g. sugar *following administration of an optimal diet*, compared with the same calculated rise during the administration of diets, consisting exclusively of respectively 100 and 300 g. sugar *following administration of a diet poor in protein*.

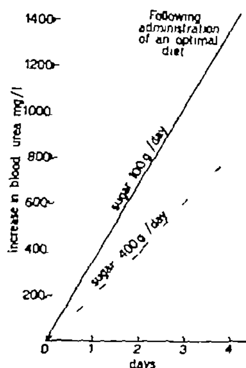


Fig. 41 – Exp V A – Calculated rise in blood urea in case of assumed acute anuria treated with diets, consisting exclusively of respectively 100 and 400 g. sugar *following administration of an optimal diet*

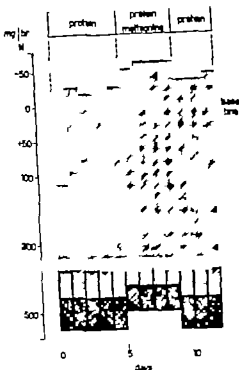


Fig. 45 Exp. VI Effect of surplus methionine on the N balance



Fig. 46 Exp. VI Effect of surplus methionine on the S balance.

ble day the catabolism of protein exceeded the intake by 10 g. In this connection the following factors may have been of influence

a) Normal diets, which ensure nitrogen equilibrium, during bed rest cause a negative nitrogen balance. This has been discussed on p. 22. Presumably the interruptions of the bed rest have been too brief to prevent the protein-catabolic influence of inactivity

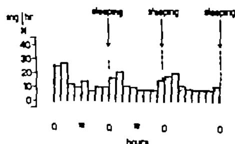


Fig. 47 Exp. VI Effect of sleep on the excretion of ammonia

♂ 21
WATER 8x300 ml/day

MILK-PROTEIN 96 gm/day
BUTTER 96 gm
SUGAR 224 gm

MILKPROTEIN
METHIONINE

1954
FEBR-MARCH 12 14 22 24 26 28 2 4

H_2O
100 ml/hr

gm/hr 0.25
0.5
FAECES + URINE TOTAL N

gm/hr 0.25 UREA N

mg/hr 25 CREATININE N

mg/hr 25 NH_3 N

mg/hr 25 P

mg/hr 25 S

+0.25
gm/hr 0
0.25
←NO FAECES→
N BALANCE (FAECES)

25
mg/hr 0
25
P BALANCE (NO FAECES)

mg/hr 0
25
S BALANCE (NO FAECES)

74
KG 72
70
68
BODY WEIGHT

Fig. 44 – Exp. VI – Effect of surplus methionine on the N and S balances. Fever on the 4th day

b) Although there is no question of directly demonstrable deficiencies in a calorically adequate diet containing 80 g. milk protein-methionine, yet the synthesis of protein is influenced better by mixtures of proteins of high biological value, of which the proportions of essential amino-acids are more in agreement with those of the body proteins.

c) The excess intake of methionine has an unfavourable effect on the uptake of other amino-acids in the body cells.

(2) The sulphur balance, also, was permanently negative and the phosphorus balance was insufficiently accurate because of the considerable faecal excretion which, due to the lack of a suitable marking technique, could not be accurately calculated per portion.

(3) The separate administration of protein and carbohydrate caused an additional loss of protein catabolites, as appears from figs. 48 and 49. If we disregard the first two days after the change of diet (REIFENSTEIN, ALBRIGHT and WELLS, 1945) we find that the additional excretion amounted to 0.9 g. nitrogen per day corresponding to 5.6 g. protein.

Two days after the original diet had been restored, the nitrogen balance was influenced more fa ourable than during the first period of the experiment. This may possibly mean a compensation of the additional loss in the period of the separate administration of the foodstuffs. However the duration of the experiment has been too short for us to determine with certainty whether the original level of excretion would ha e been reached again, and also, the growth hormone may possibly have had a disturbing influence.

(4) There was a considerable parallelism

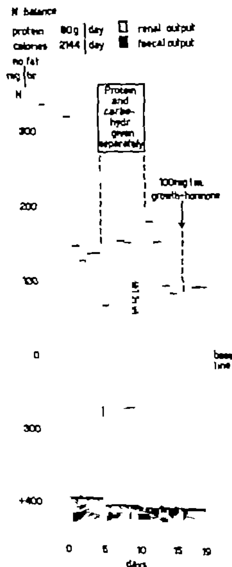


Fig. 49. Exp. VII. Effect on the N balance of separate administration of protein and sugar during the administration of fat-free diet.

between the rhythmic excretions of nitrogen and sulphur as appears from fig. 50. During this 24-hour period 8 equal portions were eaten. The differences in excretion are to be ascribed to the great oscillations in the

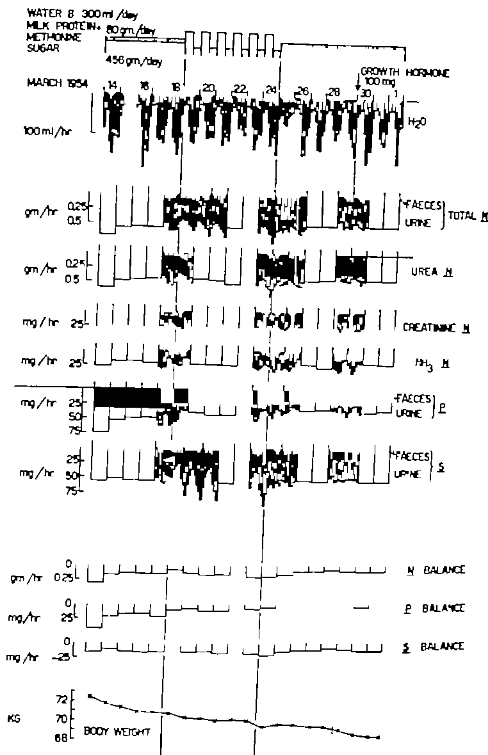


Fig 48 Exp VII Effect on the protein metabolism of separate dministration of prote and sugar during the administration of fat-free diet.

b) Although there is no question of directly demonstrable deficiencies in a calorically adequate diet containing 80 g. milk protein-methionine, yet the synthesis of protein is influenced better by mixtures of proteins of high biological value, of which the proportions of essential amino-acids are more in agreement with those of the body proteins.

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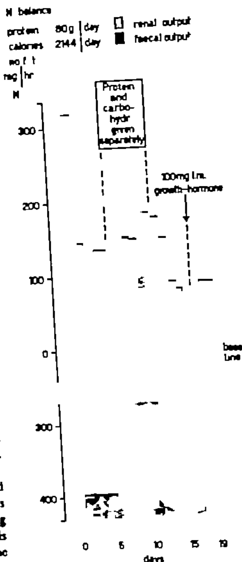


Fig. 49. Exp. VII. Effect on the N balance of separate administration of protein and sugar during the administration of fat-free diet.

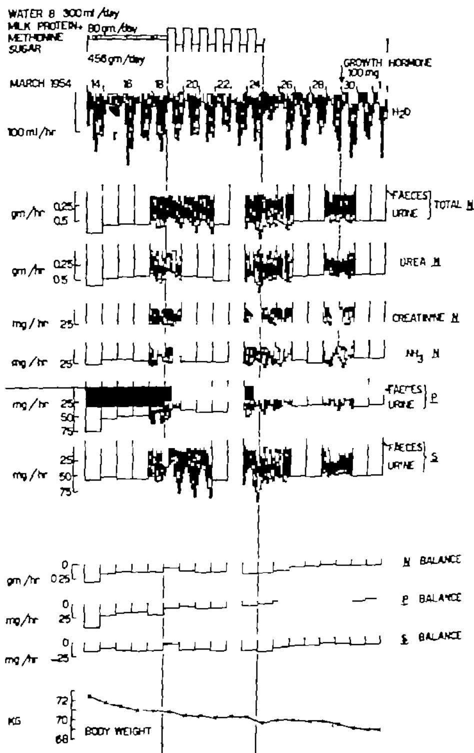


Fig. 48 Exp VII Effect on the protein metabolism of separate administration of protein and sugar during the administration of a fat-free diet.

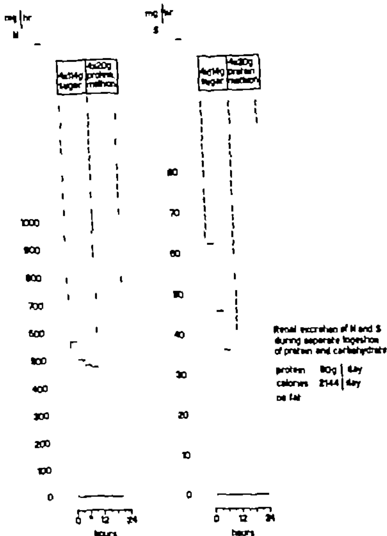


Fig 51 Exp VII Excretion of N and S, with administration of sugar in the first and protein in the second half of 24-hour period, during the administration of fat-free diet (8th day).

ceived 80 g. milk protein and 280 g. natural butter per day with minerals, water and vitamins in the same quantities as in the previous experiment. The caloric value of this carbohydrate-free diet was 2400.

During the first 6 days the food was given in 8 equal portions, with the intention subsequently to switch to separate administration of protein and fat, in order to determine whether this would lead to an extra loss of protein catabolites. However owing to the nausea it provoked the diet could not be continued for longer than 7 days.

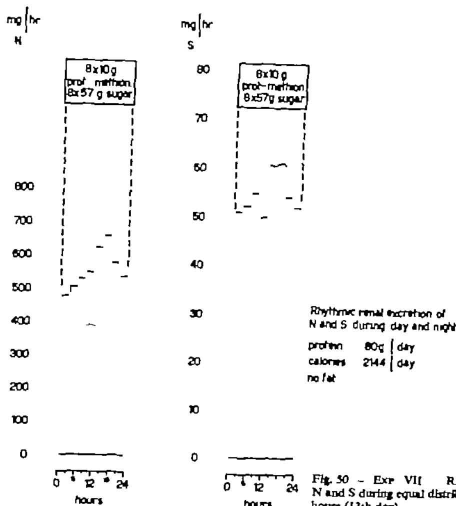


Fig. 50 – Exp VII Rhythmic excretion of N and S during equal distribution of food over 24 hours (13th day).

rhythmic excretion of water Owing to the way of life of the student in question, who had been used for years to go to bed very late and also to rise only late, the maximal excretion occurred only after 3 p m.

Fig. 51 shows the excretion of nitrogen and sulphur during one of the days with separate intake of the nutrients The excretion of protein catabolites appeared to depend upon the time scheme of the protein meals, so that the excretion pattern was in sharp contrast with the excretion during the period of 8 equal meals. Soon after the ingestion of protein the excretion of the catabolites was found to increase while there was no difference between the beginning of the increased excretion of nitrogen and sulphur From this observation it appears clearly how rapidly and how strongly the protein metabolism is influenced by administration of food exclusively consisting of protein

(5) The (animal) growth hormone had no demonstrable influence on the excretion of protein catabolites. This finding is in agreement with the investigations of Knobil and Green (1955) who demonstrated that growth hormone is to a considerable degree species-specific.

VIII – The healthy man of 22 who was the subject in the eighth experiment re-

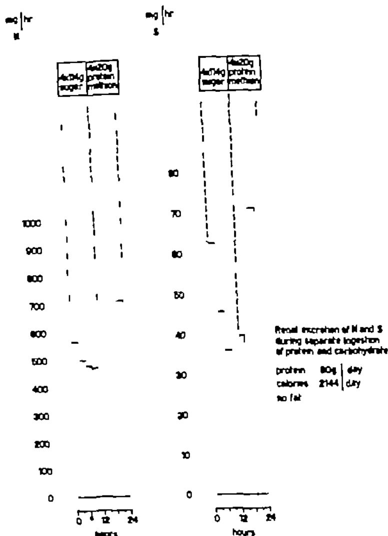


Fig. 31. Exp. VII. Excretion of N and S, with administration of sugar in the first and protein in the second half of a 24-hour period, during the administration of a fat-free diet (8th day).

ceived 80 g. milk protein and 280 g. natural butter per day with minerals, water and vitamins in the same quantities as in the previous experiment. The caloric value of this carbohydrate-free diet was 2400.

During the first 6 days the food was given in 8 equal portions, with the intention subsequently to switch to separate administration of protein and fat, in order to determine whether this would lead to an extra loss of protein catabolites. However owing to the nausea it provoked the diet could not be continued for longer than 7 days.

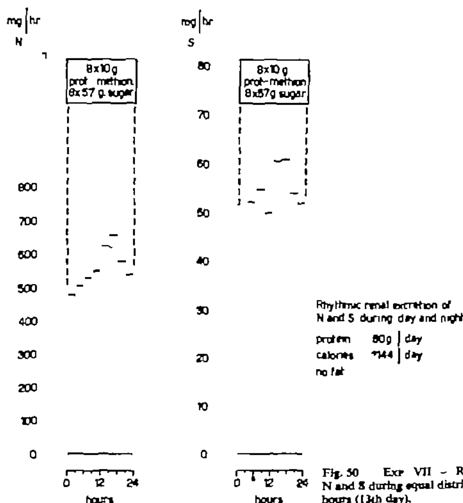


Fig. 50 Exp VII – Rhythmic excretion of N and S during equal distribution of food over 24 hours (13th day).

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VIII – The healthy man of 22 who was the subject in the EIGHTH EXPERIMENT re

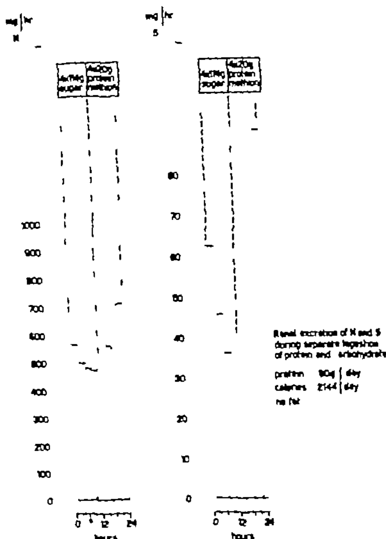


Fig. 51 Exp. VII Excretion of N and S, with administration of sugar in the first and protein in the second half of 24-hour period, during the administration of fat-free diet (9th day)

ceived 80 g. milk protein and 280 g. natural butter per day with minerals, water and vitamins in the same quantities as in the previous experiment. The caloric value of this carbohydrate-free diet was 2400.

During the first 6 days the food was given in 8 equal portions, with the intention subsequently to switch to separate administration of protein and fat, in order to determine whether this would lead to an extra loss of protein catabolites. However owing to the nausea it provoked the diet could not be continued for longer than 7 days.

♂ 22

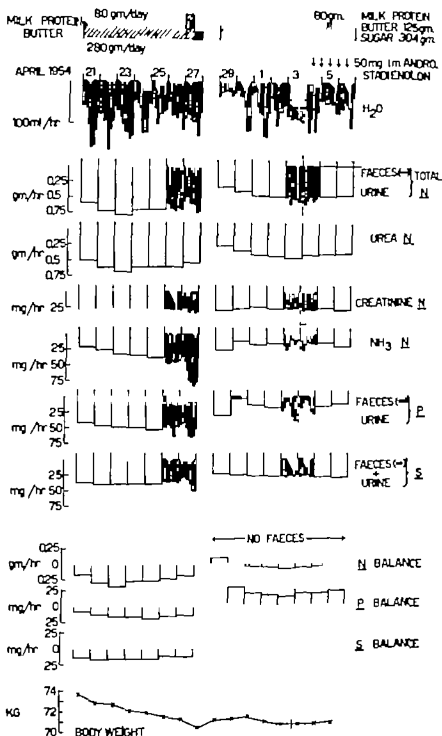


Fig. 52 Exp VIII Protein-sparing effect of isocaloric substitution of sugar for part of the fat in a carbohydrate-free diet.

After an interval of one day in which a varied diet with approximately 80 g. protein was given the patient was put on a differ ent diet which varied from the first one only in that 155 g. butter was replaced (isocalorically) by 304 g. sugar Five days after this diet had been started 6 injections, at 12-hour intervals, of 50 mg. androsta dienolone were given intramuscularly During experiments in rats this substance had been shown to have a certain protein-anabolic effect (Organon laboratory).

The findings are listed in table VIII and are shown graphically in fig. 52.

(1) In accordance with that was observed as far back as 1903 by LANDERGREN, the excretion of nitrogen was maximal on the 2nd and 3rd days of the carbohydrate-free diet (fig. 53). The relatively slight nitrogen excretion on the first day is almost certainly to be attributed to the protein-anabolic affect of the glycogen still present in the cells. Landergren explains the decreasing excretion after the third day by a certain degree of adaption of the cells, which have learned to make better use of the fats available.

After 6 days there was a pronounced acetonuria, and also a trace of diacetic acid could be demonstrated in the urine. The CO_2 combining power in the blood amounted to 17 3 m.eq HCO_3 per litre. Corresponding to this acidosis, the excretion of ammonia showed a gradual increase (fig. 52).

(2) In the course of the first week the body-weight decreased by 3.3 kg. This was not due exclusively to the increased protein catabolism. The urine secretion was greater in the period of the carbohydrate free diet than during the second period of the experiment.



Fig 53 Exp VIII The N balance during administration of diet without carbohydrates and after isocaloric substitution of sugar for part of the fat.

(3) The excretion of nitrogen, sulphur and phosphorus was considerably less during the second period. It is true that during this part of the experiment the faeces were not examined chemically but even so it is obvious that the balances were more favourable than with the sugar-free diet (figs. 52, 53). Accordingly the body-weight showed no decrease during the last period.

From the comparison of the two diets it can be concluded that for optimal protein saving carbohydrates are indispensable.

(4) As early as 12 hours after the last administration of androstadienolone the experiment had to be discontinued, so

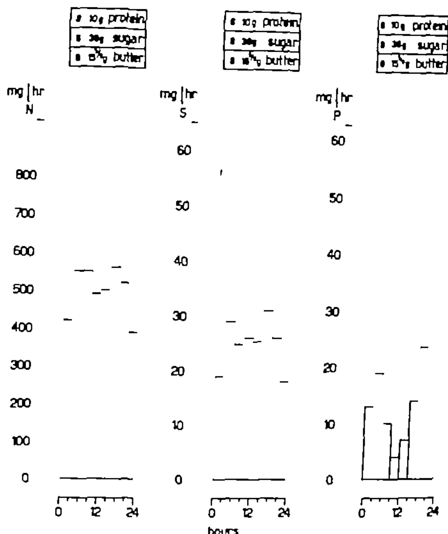


Fig. 54 – Exp VIII – Rhythmic excretion of N S and P with the urine (13th day).

that the influence of this substance on the metabolism of protein cannot be assessed.

(5) Fig. 54 shows the rhythmic excretion of nitrogen sulphur and phosphorus. From these findings it appears that the excretion of phosphorus in the course of a 24-hour period deviates considerably from the excretions of nitrogen and sulphur The metabolism of phosphorus is very complicated later on it will be discussed in greater detail

From a comparison of the graphs in fig. 54 the conclusion can be drawn that the excretion of phosphorus *is not dependent* upon protein catabolism exclusively apparently the sulphur excretion gives a more reliable impression of the metabolism of the proteins.

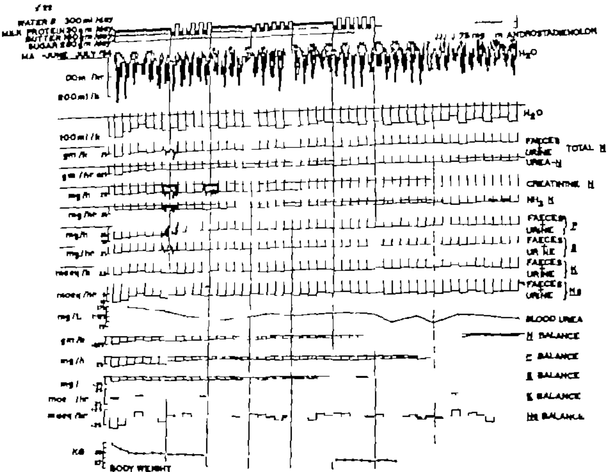


Fig. 53 Exp IX Effect of separate administration of protein and carbohydrate or fat and of androstadienolon during the administration of diet poor in protein.

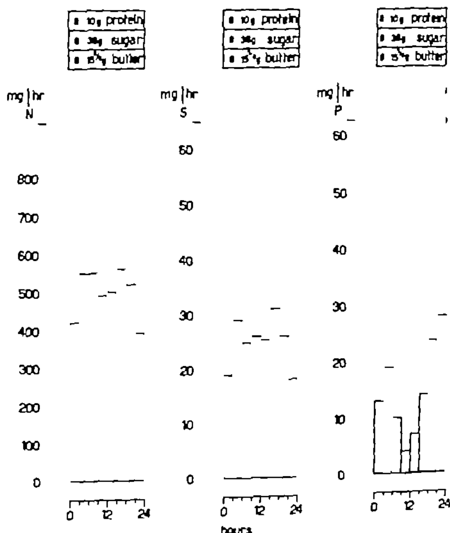


Fig. 54 – Exp VIII Rhythmic excretion of N, S and P with the urine (13th day).

that the influence of this substance on the metabolism of protein cannot be assessed.

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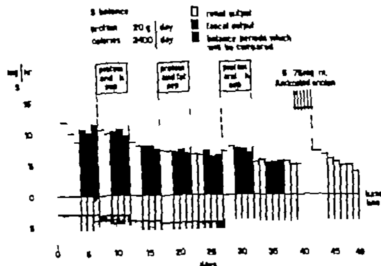


Fig. 57 Exp. IX Influence of separate administration of protein and carbohydrate or fat on the S balance during administration of diet poor in protein.

the influence of androstadienolone was again studied

The findings of this experiment are shown in table IX and fig. 55

(1) During a month prior to the beginning of the experiment, the protein in the diet had been limited to a maximum of 40 g. per day It was to be expected therefore that nitrogen equilibrium would be achieved sooner than in experiments where a greater difference exists between the protein quantities in two diets. However as can be seen in the balances of the protein catabolites, the excretion showed constant gradual decrease up to the end of the experiment.

During the last of the 7 weeks the nitrogen balance showed a minimal negativity (fig. 56). In this week, approximately 7½ g. protein was lost per 24 hours, while in the beginning the body protein decreased by approximately 16½ g. per day (from the 3rd to the 7th day of the experiment).

The urea concentration of the blood decreased from 175 to 97 mg. per litre between the 3rd and the 49th day During this period therefore the total quantity of urea in the body decreased by 70 × 0.75 × (175 − 97) mg. = 4 1 g., corresponding to an additional excretion of 41 mg. nitrogen per 24 hours, or to the catabolism of only 0.26 g. protein per day

(2) In order to determine whether as the result of the altered mode of administering food, differences develop in the excretion of the protein catabolites, two factors had to be taken into account in this experiment

a) the 2 days after an alteration had to be excluded from the evaluation (REIFENSTEIN, ALBRIGHT and WELLS, 1945), because the protein was administered during the whole 24-hour period or in 12 hours, so that the excretion of the catabolites was altered during the interval days. Therefore, in figs. 56, 57 58 and 59 the comparable periods are drawn in black. They always concern the 3 days before the

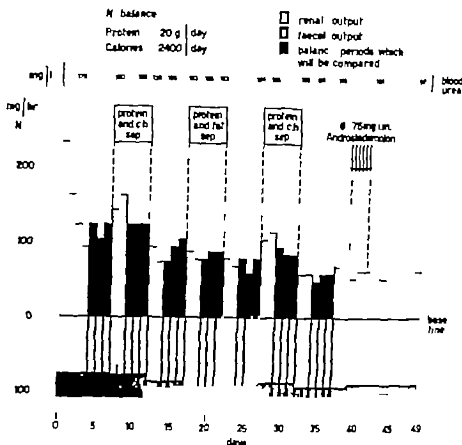


Fig. 56 Exp IX – Influence of separate administration of protein and carbohydrate or fat on the N balance during the administration of diet poor in protein.

IX – THE NINTH EXPERIMENT was carried out in a healthy man aged 22 years who for 49 days ingested per 24 hours 20 g. milk protein 160 g natural butter and 280 g. sugar plus the usual quantities of water salts and vitamins. The caloric value of the daily food intake was 2400

The main purpose of this experiment was to determine whether differences in protein-saving could be demonstrated when carbohydrate or fat was given together with protein or separately A small quantity of protein was prescribed so as to imitate as much as possible the diet usually given to patients with uraemia. During the first 7 days 8 equal portions were given containing protein sugar and butter During the subsequent 5 days the butter was consumed in 8 equal portions, and the protein in 4 equal portions during the first half of a 24-hour period and the sugar in the same way during the second half Subsequently the diet of the first week was repeated for 5 more days, followed by another 5-day period during which the sugar was divided into 8 equal portions with the protein given in 4 equal portions during the first half and the butter in the same manner during the second half of the day This was again followed by a period of 5 days with equal distribution of the foodstuffs, after which the second period was repeated for 5 days, with separate administration of sugar and protein During the subsequent period when the food was once more administered in 8 equal portions

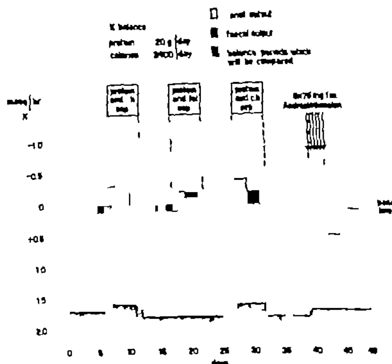


Fig. 59 Exp. IX. Influence of separate administration of protein and carbohydrate or fat on the K balance during the administration of diet poor in protein.

tration. The line which connects the average excretion during the control periods, shows an angle, which decreases towards the end of the experiment (with the exception of the last potassium excretion) which means that the rate of the daily improvement of the balances decreased in the course of the experiment.

During the periods of separate administration of protein and sugar the additional excretions of nitrogen were 19 and 24 g. respectively corresponding to 2.85 and 3.6 g. protein respectively per 24 hours. The extra excretion amounted during the period of separate fat feeding to 3 mg. nitrogen per hour corresponding to 0.45 g. protein per day. The extra loss of sulphur during the separation of protein and sugar

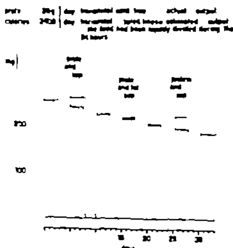


Fig. 60. Exp. IX. N excretion increased by administering protein and carbohydrate separately. Absence thereof under separate administration of protein and fat.

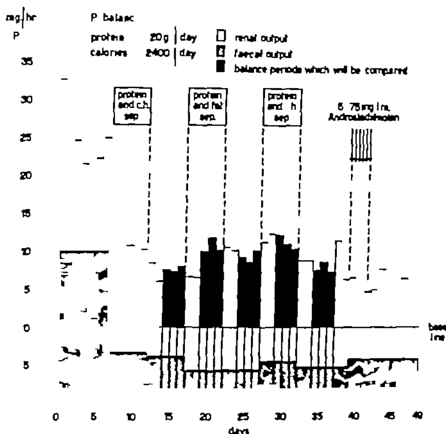


Fig. 58 – Exp IX – Influence of separat administration of protein and carbohydrate or fat on the P balance during the administration of diet poor in protein.

change of diet and the 3rd 4th and 5th day afterward

b) in view of the fact that the excretion of the protein catabolites decreased in the course of the experiment, a correction had to be made to eliminate this time factor

Owing to the very regular decrease, appearing from the excretions during the periods of equally distributed food, it proved possible to interpolate for the remaining periods, obtaining as the result the excretion which would have been observed had the food been administered in 8 equal portions all the time.

In figs. 60 61 and 62 the excretions of nitrogen sulphur and potassium are shown taking into account the two factors mentioned above This could not be done for phosphorus because of the disturbing influence of the considerable faecal excretion of phosphorus during the first period which perhaps had been insufficiently separated from the excretion prior to the experiment. In the figures, the actual excretion is shown by blocks, bordered by continuous horizontal lines, whereas the horizontal dotted lines have been drawn after interpolation between the two adjacent values of excretion during periods in which the food was distributed equally over the 24-hour period. The first 2 days of each period have not been used in the evaluation The difference in height between the continuous and the dotted horizontal lines indicates the loss or gain during the periods with separate adminis-

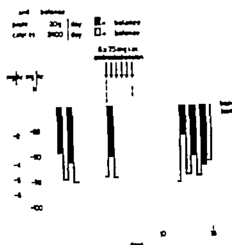


Fig. 63 Exp IX N and S balance under the influence of androstadienolon.

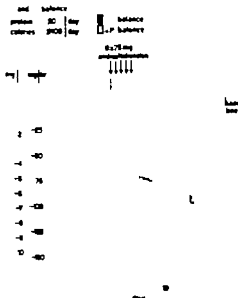


Fig. 64 Exp IX N and P balance under the influence of androstadienolon.

in doses of 75 mg. per injection at 12-hour intervals.

Figs. 63 64 and 65 show in comparison the excretions of nitrogen and sulphur nitrogen and phosphorus, and nitrogen and potassium. It appears from fig. 63 that the administration of androstadienolone brought about an increase of the excretion of sulphur whereas the excretion of nitrogen was hardly affected.

The increased excretion of sulphur was associated with a decrease of the excretion of phosphorus and potassium, as appears from figs. 64 and 65

These observations will be once more discussed in separate chapters.

X The results of the TENTH EXPERIMENT are shown in table X and in fig. 66. The subject was a healthy man 20 years old, who over 20-day period received per day in 8 equal portions 80 g. milk protein, 280 g. sugar 120 g. of natural butter in addition to the usual quantities of water salts, and vitamins. The caloric value was

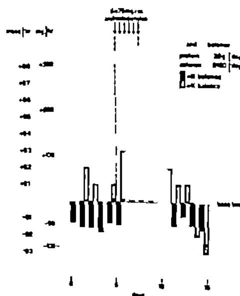


Fig 65 Exp IX N and K balance under the influence of androstadienolon.

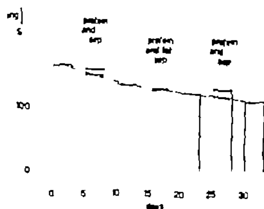


Fig. 61 – Exp IX – The S excretion follow the pattern of the N excretion (fig. 60) during separat administration of protein and carbohydrat or fat.

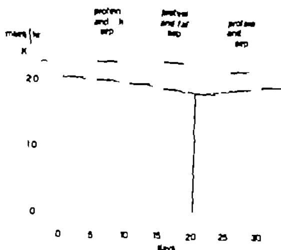


Fig. 62 – Exp IX The K excretion also increases if protein nd fat are administered separat ly

was 0.9 and 1.2 mg. per hour respectively which roughly corresponds to 2.2 and 2.9 g. protein per 24 hours. During the separate administration of protein and fat there was even a slight gain which amounted to 0.2 mg. sulphur per hour corresponding to 0 5 g. protein per 24 hours.

The potassium excretion was higher not only during the periods with separate intake of protein and carbohydrate, but also during the period when protein and fat were given separately The extra excretion amounted in the first case to 0.28 and 0.29 m eq potassium per hour respectively while the change of the fat diet brought about an additional loss of 0 41 m.eq potassium per hour

Although no accurate conclusions can be drawn from the phosphorus balance (fig. 58) it still shows that phosphorus, like potassium, was excreted to a higher degree during the period of separate administration of protein and fat It has been stated previously that the excretion of phosphorus cannot simply be used as a measure of the protein catabolism. The same can be said of potassium. The increased excretion of phosphorus and potassium during the separate administration of protein and fat therefore does not necessarily mean that more protein was disintegrated. The absence of an additional excretion of nitrogen and sulphur under these conditions constitutes an indication that the loss of phosphorus and potassium was the consequence of other factors.

It appears from the above that the separate administration of protein and carbohydrate leads to an increased excretion of nitrogen and sulphur whereas this time factor is not valid for fat. The proportion in which nitrogen and sulphur are excreted to a greater degree constitutes a strong indication that more protein is actually being disintegrated

(3) In the last period of the experiment the influence of androstadienolone on the excretion of protein catabolites was studied. It was given in 6 intramuscular injections



Fig. 63 Exp IX N and S balance under the influence of androstadienolon.



Fig. 64 Exp IX N and P balance under the influence of androstadienolon.

in doses of 75 mg. per injection at 12-hour intervals.

Figs. 63 64 and 65 show in comparison the excretions of nitrogen and sulphur nitrogen and phosphorus, and nitrogen and potassium. It appears from fig. 63 that the administration of androstadienolone brought about an increase of the excretion of sulphur whereas the excretion of nitrogen was hardly affected.

The increased excretion of sulphur was associated with a decrease of the excretion of phosphorus and potassium, as appears from figs. 64 and 65

These observations will be once more discussed in separate chapters.

X The results of the TENTH EXPERIMENT are shown in table X and in fig. 66. The subject was a healthy man 20 years old, who over a 20-day period received per day in 8 equal portions 60 g. milk protein, 280 g. sugar 120 g. of natural butter in addition to the usual quantities of water salts, and vitamins. The caloric value was

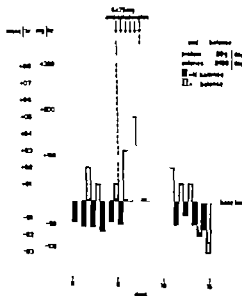


Fig 65 Exp IX N and K balance under the influence of androstadienolon.

♂ 20
WATER 8 x 300 ml/day
MILK PROTEIN 80 gm/day
BUTTER 125 gm/day
SUGAR 280 gm/day

90 mg i.m. MAD
FEBR. 55

100 ml/hr
200 ml/h
H_2O

100 ml/hr
H_2O

gm/hr
FAECES + URINE } TOTAL N

gm/hr
UREA N

mg/h
CREATININE N

mg/hr
NH_3 N

mg/h
FAECES + URINE } P

mg/h
FAECES + URINE } S

m eq/hr
FAECES + URINE } K

m eq/h
FAECES + URINE } Na

mg/L
BLOOD UREA

gm/h
N BALANCE

mg/hr
S BALANCE

mg/hr
P BALANCE

m eq/hr
K BALANCE

m eq/h
Na BALANCE

KG
BODY WEIGHT

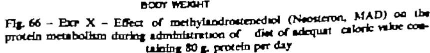


Fig. 66 – Exp X – Effect of methylandrostenediol (Neosteron, MAD) on the protein metabolism during administration of diet of adequat caloric value containing 80 g. protein per day

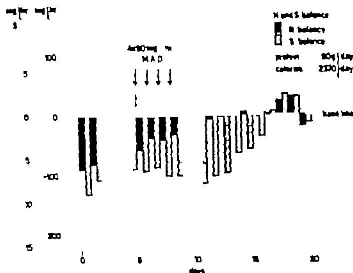


Fig 67 Exp X Disparity in the N and S balances under the influence of MAD during the administration of diet containing 80 g protein per day

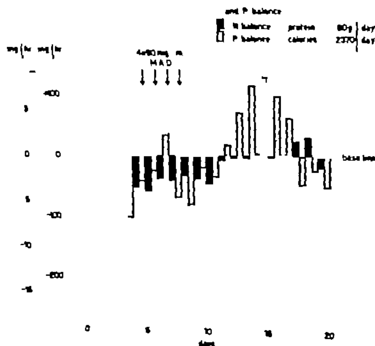


Fig 68 Exp X Disparity in the N and P balances under the influence of MAD, during the administration of diet containing 80 g protein per day

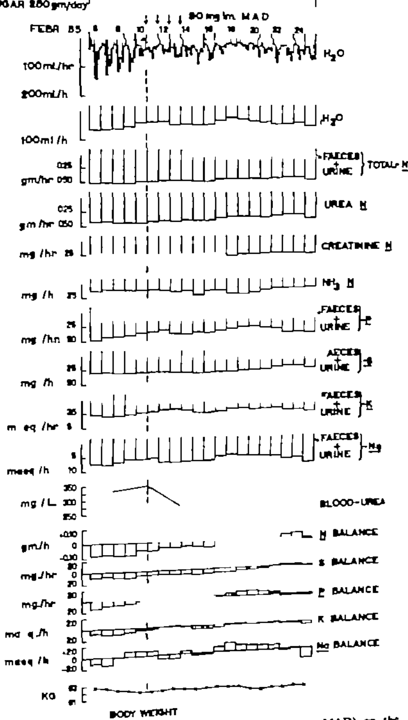


Fig. 66 – Exp X Effect of methylandrostenediol (Neosteron, MAD) on the protein metabolism during administration of a diet of adequat caloric value containing 80 g. protein per da

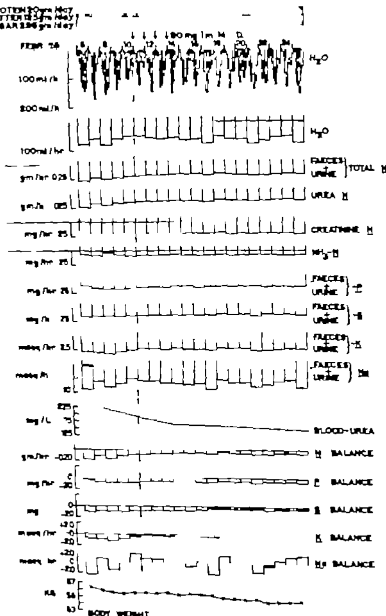


Fig 70 Exp XI Effect of methylandrostenediol (Neostenon, MAD) on the protein metabolism during administration of diet of adequate caloric value containing 20 g protein per day

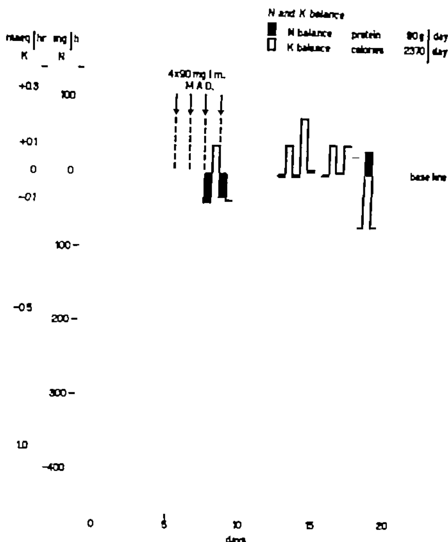


Fig. 69 – Exp. X – Disparity in the N and K balances under the influence of MAD during the administration of diet containing 80 g. protein per day

2370 per day. Five days after the beginning of the test the subject received one intramuscular injection of 90 mg. methylandrostenediol (MAD) per day for 4 days.

In addition to the determinations mentioned for previous experiments the urinary excretion of creatine was also examined.

(1) In fig. 67 the nitrogen and sulphur balances are shown. MAD caused a distinct decrease of the nitrogen excretion whereas the excretion of sulphur initially increased and thereafter for several days still showed a relatively less pronounced decrease than the excretion of nitrogen. This discrepancy between nitrogen and sulphur under the influence of anabolic steroids has also been observed in previously described experiments.

(2) In figs. 68 and 69 it can be seen that the excretions of phosphorus and potassium under the influence of MAD decreased more than the excretion of nitrogen. The effect of MAD is twofold. Soon after the administration of the steroids a retention of phosphorus and potassium occurred

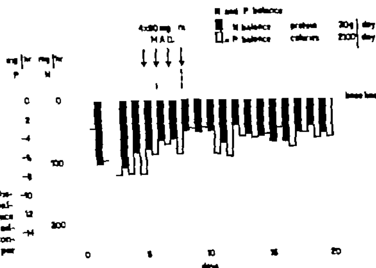


Fig. 72. Exp. XI. Disparity in the N and P balances under the influence of MAD during the administration of diet containing 20 g. protein per day

day in addition to 296 g. sugar and 125 g. natural butter. The caloric value was 2100 per day. In all other respects this test was the same as the previous one. The dosage of MAD was also the same.

The findings are listed in table XI and shown graphically in fig. 70.

(1) In this case also the nitrogen balance showed a distinct negativity until the last, the 20th day. The excretion exceeded the intake during the last 3 days by an average of 52 mg. nitrogen per hour corresponding to 7.8 g. protein per 24 hours. The causes of the relatively large excretion of protein catabolites have been mentioned before.

The difference between the blood urea concentrations at the beginning and at the end of the experiment was too small to be of significance in the evaluation of the degree of protein catabolism.

(2) The protein saving brought about by MAD was much less than with the high-protein diet. This is in agreement with earlier observations which are mentioned in the literature (fig. 71).

(3) It appears further from fig. 71 that the excretion of sulphur after the administration of MAD at first did not decrease at all. Under the influence of MAD therefore the urine was temporarily relatively rich in sulphur with this low-protein diet as well, although the differences between nitrogen and sulphur were more pronounced with the diet containing 80 g. protein per day.

(4) The disparity in the nitrogen balance and the balances of phosphorus and potassium under the influence of MAD is less pronounced than with the high-protein diet (figs. 72 and 73).

(5) Although the excretion of sodium was not very regular, it can be seen from fig. 70 that MAD caused no retention of importance.

The excretion of water also was not influenced, nor was the body-weight affected to any significant degree.

(6) The urine contained no creatine.

XII. – In the TWELFTH EXPERIMENT a healthy man aged 19 years received a

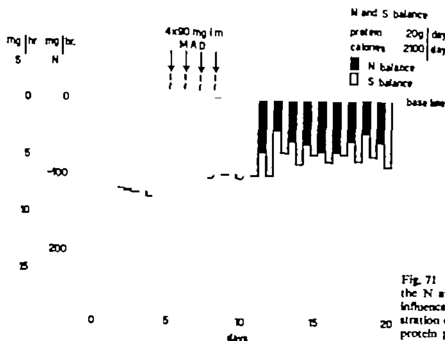


Fig. 71 Exp XI Disparity in the N and S balances under the influence of MAD during administration of diet containing 20 g. protein per day

followed by an additional excretion which was possibly of compensatory nature Thereafter a greater retention of longer duration was observed which coincided with the period of nitrogen saving. It was found however that this second saving of phosphorus and potassium was also greater in proportion to the nitrogen than might be expected from the composition of the body protein The additional retention of phosphorus and potassium was again followed by a period of relatively increased excretion

(3) MAD caused a slight retention of water and sodium (fig. 66) which persisted for only a few days.

(4) Creatine could not be found in any of the specimens of urine. QUERIDO KASSENAAR, SCHUURS and SELDENRATH (1952) have observed a distinct creatinuria after treatment of a patient with hypogonadism with 75 mg. MAD per day for 12 days. They quote HOBERMAN SIMS and ENGSTROM (1948), who were able to demonstrate that steroids with a 17 methyl group in the molecule stimulate the production of creatine. This may go so far that the muscles have insufficient capacity to take up all the creatine, so that a part is excreted Moreover as the consequence of this extra uptake of creatine more phosphorus will be incorporated in the muscles as well. It is to this that Querido et al attribute the phenomenon that the retention of phosphorus in proportion to nitrogen after administration of MAD is greater than corresponds to the composition of body protein

It will appear from experiments to be described later that anabolic steroids without 17 methyl group, which do not stimulate the production of creatine nevertheless bring about an extra retention of phosphorus and potassium

XI – THE ELEVENTH EXPERIMENT also was concerned with the influence of MAD on the excretion of protein catabolites.

The subject, a man of 21 however was given not 80 but 20 g. milk protein per

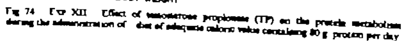


Fig 74 Exp XII Effect of testosterone propionate (TP) on the protein metabolism during the administration of diet of adequate caloric value containing 80 g protein per day

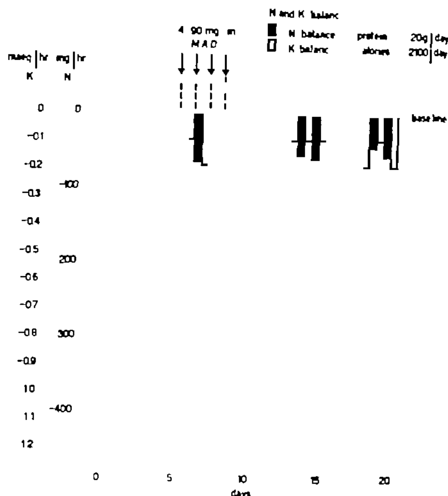


Fig. 73 – Exp XI Disparity in the N and K balances under the influence of MAD during the administration of diet containing 20 g. protein per day

diet which consisted of 80 g. milk protein, 360 g. natural butter and the usual quantities of water salts and vitamins The food was given in 8 equal portions at 3-hour intervals. The caloric value was 2690 per day

Nine days after the beginning of the test 250 mg. testosterone propionate (TP) was administered intramuscularly together with 100 units hyaluronidase 8 days later only the hyaluronidase was injected The rapidly absorbing TP was selected in an attempt to obtain a clearly demonstrable retention of protein catabolites. This was also the purpose of the administration of the hyaluronidase This substance causes a considerable acceleration of the absorption of aqueous solutions, but the protein-anabolic effect of the TP dissolved in oil did not become noticeable sooner than has been observed by others.

The findings are listed in table XII and recorded graphically in fig. 74

(1) In spite of a relatively normal caloric value and protein content the nitrogen balance was persistently negative during the experiment. The causes of this phenomenon have previously been described.

(2) On the first day after administration of TP an increased excretion of nitrogen was observed. This additional excretion

of nitrogen during the first 24 hours after administration of anabolic steroids has been described more often (see the review of the literature).

Starting on the second day there was a distinct retention of nitrogen which lasted 5 days (fig. 75). In this period, a total of approx. 59.0 g. protein was saved. The maximal retention, 17.1 g., was caused on the second day.

(3) It appears from the proportion between the nitrogen and sulphur balances that in this case also initially relatively much sulphur was excreted, whereas thereafter probably because of compensation, relatively more sulphur than nitrogen was retained (fig. 75).

(4) Under the influence of TP once more phosphorus and potassium were retained relatively more markedly than nitrogen (figs. 76 and 77).

A clearly diphasic retaining effect could not be observed. This may perhaps be attributed to the well known fact that TP acts more quickly than MAD so that the two saving influences perhaps partially overlapped each other.

There is a great similarity of the effect of MAD and of TP on the balances of the protein catabolites, particularly when in evaluation the difference between the absorption rates is taken into account.

(5) In contrast to the absence of a distinct retention of water and of sodium after administration of MAD it was found, as appears from fig. 74 that 250 mg. TP did bring about an important decrease of the excretion of water and sodium, so that a transient increase of the body-weight could be observed. The retention of water and sodium lasted a shorter time than that of phosphorus and potassium.

XIII – THE THIRTEENTH EXPERIMENT was carried out in a healthy man aged 21 years who daily received 20 g. milk protein, 360 g. sugar 125 g. butter and the usual quantities of water salts and vitamins, always divided over 8 equal portions given at 3-hour intervals. This diet yielded 2450 calories per 24 hours.

Nine days after the beginning of the test 250 mg. testosterone propionate (TP) was administered intramuscularly together with 100 units hyaluronidase 8 days later only the hyaluronidase was injected. In other words this experiment is practically identical to the previous one. The quantity of protein in the diet was the only factor with a difference, so as to enable us to compare the anabolic effect of TP during the administration of high-protein and low protein diets.

The findings are listed in table XIII and shown graphically in fig. 78.

(1) The negative nitrogen balance showed a gradual improvement in the course of the experiment. The pronounced decrease of the nitrogen excretion which characterizes the switch from a high-protein to a low-protein diet did not occur in this case, because the quantity of protein in the diet had already been restricted beforehand.

(2) Under the influence of TP a marked nitrogen retention occurred beginning one day after the injection and lasting 4 days (fig. 79). If we take into account the spontaneous, gradual decrease of the nitrogen excretion we can calculate that in the first 5 days after the injection approximately 26.0 g. protein was saved.

On the 4th day after administration of TP the amount was 9 g. It appears from these figures that the maximal retention with the low-protein diet occurred 2 days

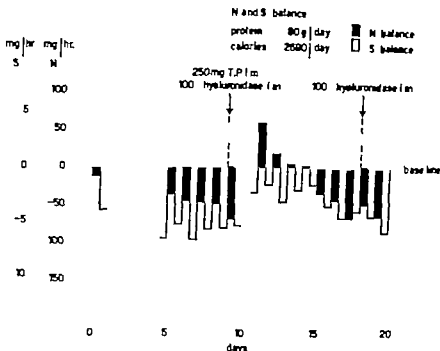


Fig. 75 – Exp XII – Disparity in the N and S balances under the influence of TP during the administration of diet containing 80 g. protein per day

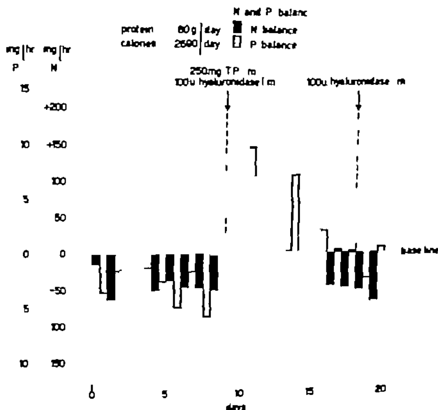


Fig. 76 Exp XII Disparity in the N and P balances under the influence of TP during the administration of diet containing 80 g. protein per day

Fig 73   Exp XIII   Effect of testosterone propionate (TP) on the protein metabolism during the administration of diet of adequate calorie value containing 20 g protein per day

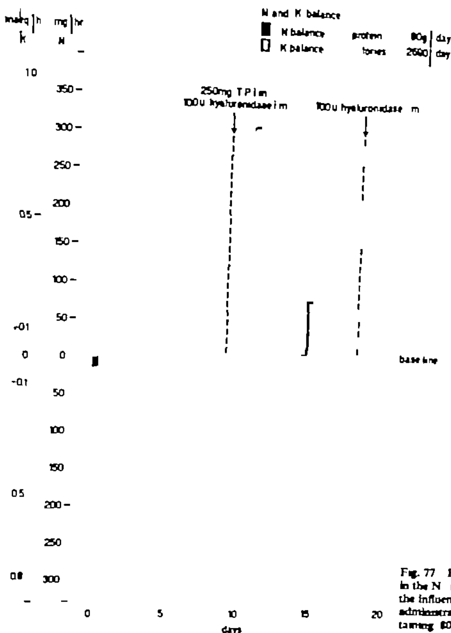


Fig. 77 Exp XII Disparity in the N and K balances under the influence of TP during the administration of diet containing 80 g. protein per day

later than with the high protein diet. With the diet containing 80 g. protein the saving of protein under the influence of TP was approximately twice as much as with the diet containing 20 g. protein. The average decrease in percentages of the nitrogen excretion during the 2nd to the 6th day inclusive after administration of TP was in both experiments approximately 20 per cent, however The same was true of the decrease of the quantity of the variable nitrogen, originating from urea and ammonia

(3) The improvement of the sulphur balance in this case also initially lagged behind that of nitrogen (fig. 79). The relatively high sulphur excretion changed after 5 days into a relatively low excretion

(4) The influence of TP on the phosphorus and potassium balances again proved to

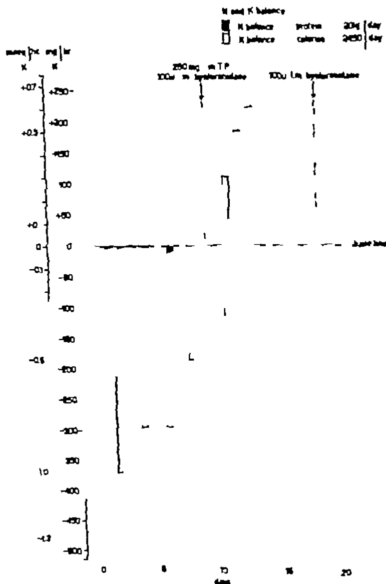


Fig. 81 Exp. XIII Discrepancy in the N and K balances under the influence of TP during the administration of diet containing 20 g protein per day

be more pronounced than the effect on the nitrogen balance (figs. 80 and 81).

(5) In this experiment also a short lasting retention of water and sodium after administration of TP with a corresponding temporary increase of the body-weight, was observed.

(6) Fig. 82 shows the daily excretion of creatinine during the XIIth and the XIIIth experiment. It appears from this figure

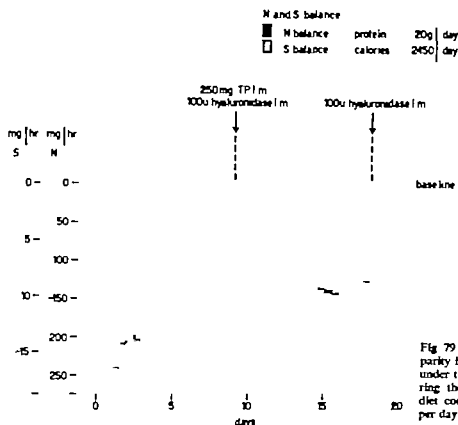


Fig 79 Exp XIII Disparity in the N and S balances under the influence of TP during the administration of a diet containing 20 g. protein per day

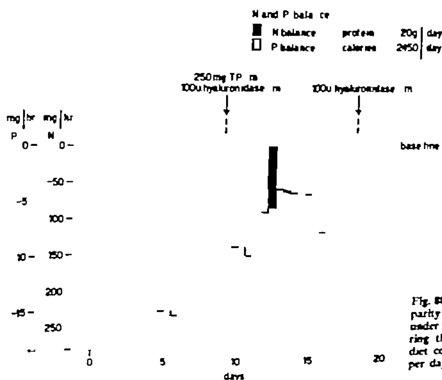


Fig. 80 Exp XIII Disparity in the N and P balances under the influence of TP during the administration of diet containing 20 g protein per day

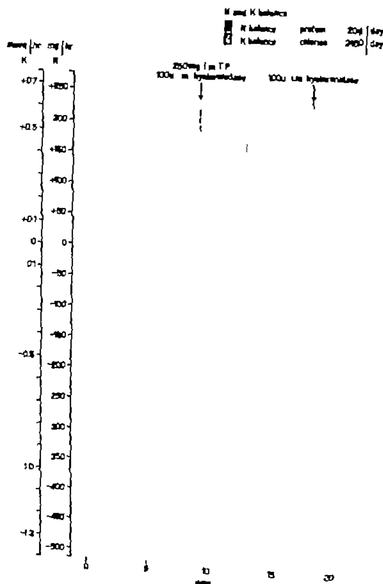


Fig. 81 Exp. XIII Disparity in the N and K balances under the influence of TP during the administration of diet containing 20 g. protein per day

be more pronounced than the effect on the nitrogen balance (figs. 80 and 81).

(5) In this experiment also a short lasting retention of water and sodium after administration of TP with a corresponding temporary increase of the body-weight, was observed.

(6) Fig. 82 shows the daily excretion of creatinine during the XIIth and the XIIIth experiment. It appears from this figure

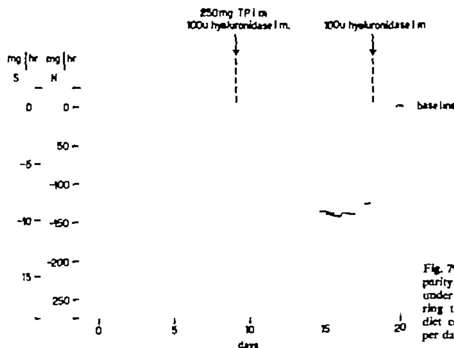


Fig. 79 Exp XIII Disparity in the N and S balances under the influence of TP during the administration of a diet containing 20 g protein per day

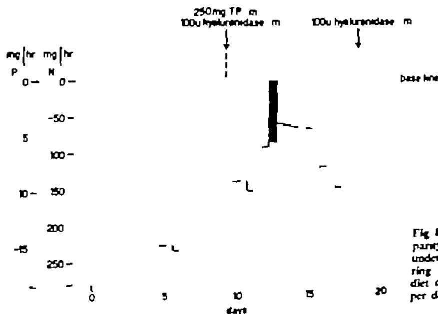


Fig. 80 E. XIII Disparity in the N and P balances under the influence of TP during the administration of diet containing 20 g protein per day

Fig. 13 Exp. XIV Effect of nor-androstenolone phenylpropionate (Durabolin, NAPP) and of extra NaCl on the protein metabolism during the administration of a diet of adequate caloric value containing 20 g. protein per day

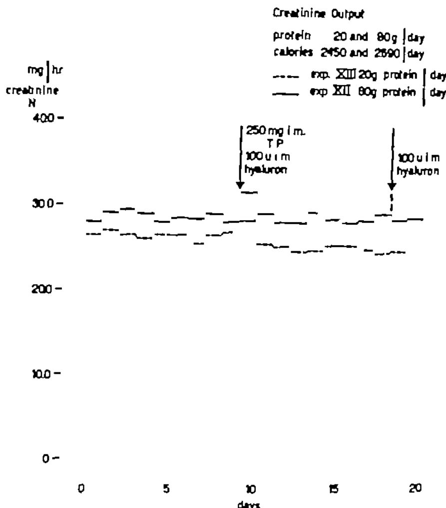


Fig. 82 Exp XII & XIII – Influence of TP on the excretion of creatinine in two subjects on diets of adequat caloric value containing 80 and 20 g. protein per day respectively

that the excretion of creatinine did not change in the course of the XIIth experiment whereas during the low protein diet a gradual decrease occurred In either case an increased excretion of creatinine was only found on the first day after administration of TP The presence of creatine could not be demonstrated in these portions of urine

A further discussion of the excretion of creatinine will be presented later on

XIV – THE FOURTEENTH EXPERIMENT was carried out in a healthy man aged 20 years, who was given a daily diet of 20 g milk protein 368 g. sugar 125 g. natural butter and the usual quantities of water salts and vitamins The food was given in 8 equal portions, at 3-hour intervals. The caloric value was 2450 per day

Nine days after the beginning of the test 125 mg. nor testosterone phenylpropionate (NAPP) was injected intramuscularly and 7 days later 20 g. sodium chloride per day was added to the diet for 6 days.

Three days later the experiment was ended On this occasion the excretions were also examined for calcium.

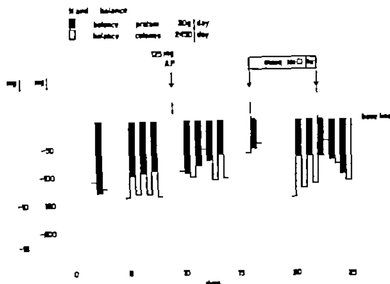


Fig. 86 Ex XIV Effect of NAPP and of extra NaCl on the N and P balances during the administration of a diet of adequate caloric value containing 20 g. protein per day

curves one must take into account the fact that NAPP in the XIVth experiment has been administered during a period of spontaneous decrease of the excretion. It seems obvious to explain the difference in the degree of nitrogen saving on the basis of the difference in age between the two test subjects. The anabolic effect was less clearly discernable in the man aged 20 years, because the natural production of androgenic substances was presumably greater than in the subject of the second experiment who was 45 years old. MIGEON KELLER, LAWRENCE and SHEPARD (1957) determined the quantity of dehydroepiandrosterone and androsterone in the plasma of many healthy persons. The greatest quantities of these important 17 ketosteroids were encountered in men between the ages of 23 and 39 and in women of the same ages the quantities were only slightly less. After the 40th year however both sexes presented a rapid and distinct decrease.

(3) The sulphur balance again showed the same characteristics as in earlier experiments after administration of anabolic steroids (fig. 85). The increased sulphur excretion caused us to ask the manufacturer of NAPP whether perhaps a sulphur containing substance had been added to the preparation, which proved, however not to be the case.

(4) The excretion of phosphorus and potassium under the influence of NAPP decreased relatively more strongly than the nitrogen excretion (figs. 86 and 87). The effect on the balance of phosphorus and potassium during the second week after the administration of NAPP could not be evaluated because of the additional excretion which had been brought about by the addition of 20 g. sodium chloride to the diet.

The findings are listed in tabl XIV and presented graphically in fig. 83

(1) The excretion of nitrogen decreased gradually with this low protein diet as well The rapid phase of this decrease did not occur because the subject had been put on a low protein diet beforehand.

(2) Under th influence of NAPP a slight nitrogen retention developed, the duration of which could not be determined, because the addition of sodium chlond may also have had a protein-saving effect. In order to make possible a comparison with the nitrogen retention caused by NAPP during the first part of the second experiment, the nitrogen balance of the XIVth test has been drawn on th ame scale as in fig. 8 and the excretion has also been calculated per 3-day period (fig. 84). It can be seen that the nitrocen saving in this instant was less than in the second experiment. In comparing the 2

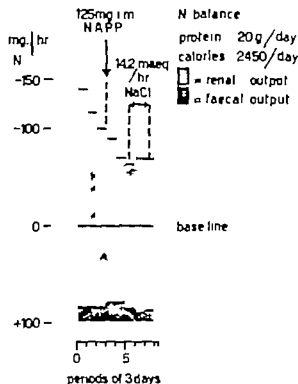


Fig. 84 – Exp XIV Effect of NAPP and of extra NaCl (14.2 m.eq per hour) on the N balance f a 20-year-old man during the administration of diet poor in protein. Compare with fig. 8 for the same test in a 42-year-old man.

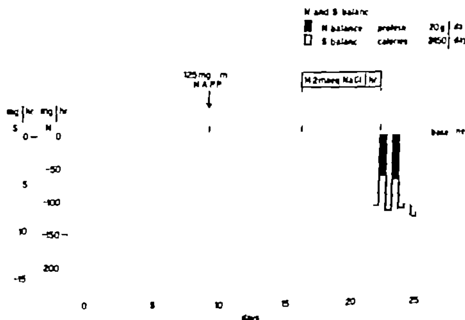


Fig. 85 – Exp XIV Effect of NAPP and of extra NaCl on the N nd S balances during the administration of diet of adequat caloric value containing 70 g. protein per day

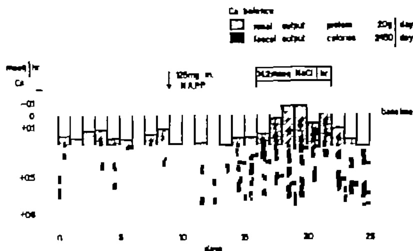


Fig. 88 Exp. XIV Effect of NAPP and of extra NaCl on the Ca balance during the administration of diet of adequate caloric value containing 20 g. protein per day

a diet already containing 9 1 g. NaCl caused a slight increase of the body weight only during the first 2 days (fig. 83).

b) In accordance with this, the sodium balance was positive for 2 days only after the administration of extra salt was discontinued, sodium equilibrium was regained within one day (fig. 89).

c) The excretion of nitrogen was probably hardly influenced (fig. 85). Apparently this is not in agreement with the data from the literature mentioned previously. However SANDERSON (1954) and LEAF and COUTER (1949) gave 3 times and 1½ times as much sodium, respectively and compared the protein catabolism with a period in which no sodium had been administered.

d) The excretions of phosphorus and particularly of potassium showed a considerable increase i the course of the high-

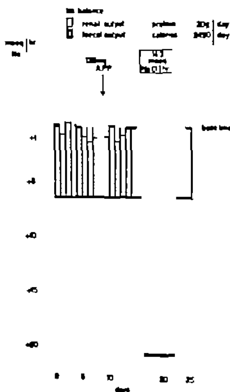


Fig. 89 Exp. XIV Effect of NAPP and of extra NaCl on the Na balance during the administration of diet of adequate caloric value containing 20 g protein per day

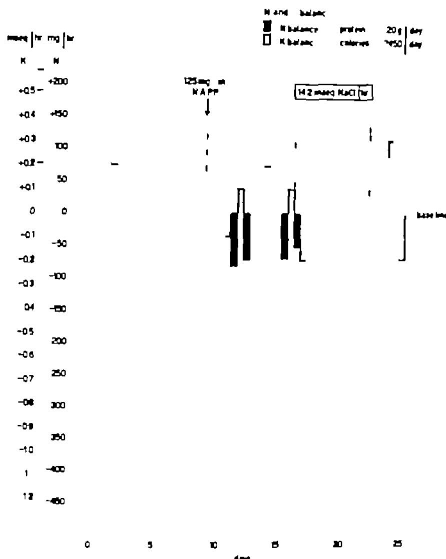


Fig. 87 Exp. XIV – Effect of NAPP and of extra NaCl on the N and K balances during the administration of diet of adequate caloric value containing 20 g. protein per day

(5) The calcium balance was slightly positive and under the influence of NAPP a little more calcium was retained (fig. 88).

(6) In spite of the standardized conditions under which the experiments VI to XIV conclusive, were carried out, the daily variations of the excretion of sodium proved to be rather large. Nevertheless fig. 89 shows sufficiently clearly that the sodium balance under the influence of NAPP underwent no change of significance. The almost complete absence of water and salt retention after administration of NAPP is of great significance for the practical value of this preparation.

(7) The period between the administration of NAPP and the beginning of the high-salt diet was too brief so that the balances of the protein catabolites are difficult to evaluate. However some cautious conclusions may be drawn from the results, if w also consider the observations in the IIIrd experiment.

a) The addition of 20 g. NaCl per day to

this period the patient received a blood transfusion of 1½ litre which caused no increase of the blood urea concentration. Toward the end of March 1956 he was discharged. The general condition at that time was reasonably good. The blood pressure was 140/90 on the average. The urea clearance was 4.5 / with a blood urea concentration of 863 mg. per litre. In April the patient was able to resume his daily activities.

Control examinations in the outpatient department revealed that the renal function continued to decrease gradually For the persistent anaemia a bloodtransfusion had to be administered several times. Toward the end of November the urea clearance was 4 / and the blood urea concentration was 1485 mg./l At that time, nausea made it impossible to maintain the diet any longer so that another diet was prescribed, which contained 15 g. protein. From November 29 to December 21 1956 this diet was given under standardized conditions which will be described later

In January and February 1957 the patient felt reasonably well He was able to do some light work. Towards the end of January the urea clearance was 3.5 / with a blood urea concentration of 1041 mg. per l. The blood pressure was 180/120 no pronounced cardiovascular abnormalities could be demonstrated. The electrolyte concentrations in the plasma and the level of albumin and total protein in the serum were still normal at that time.

On March 8 1957 the condition was found to ha e deteriorated considerably which was *mainly due to cardiac insuf* ficiency brought about by pericarditis. There was a low pulse pressure and symptoms of beginning pulmonary oede ma. The patient suffered much from vomit ing for which chlorpromazine was only partly efficacious. The blood urea concentration increased in 10 days from 2075 to 3730 mg./l. The patient died on March 24 1957

Post mortem examination (Dr R. van Dam) revealed not only the renal affection, but also exudative pericarditis. The much shrunken kidneys exhibited hyalinization of practically all glomeruli and atrophy of many tubuli, probably the consequence of glomerulo-nephritis. There was how ever also considerable sclerotic alteration of arteries and arterioles.

Epicrisis this man of 32 has suffered from an increasing renal insufficiency brought about by chronic glomerulo-nephritis. In all probability the addition al vascular process hastened the progress of the renal condition

With the aid of diets, it proved possible, up to less than one month prior to death, to keep the blood chemistry almost normal with the exception of a refractory anaemia for which transfusions had to be given at shorter and shorter intervals. The complaints probably were almost exclusively due to the anaemia. Following each blood transfusion the patient once more became able to do his work as a carpenter

In table XV a number of data from the case history are listed.

XV THE FIFTEENTH EXPERIMENT lasted from February 9 to March 24 1956, inclusive. The diet of the uraemic patient contained 18 g. protein, 376 g. carbohydrate, and 130 g. fat per day

The caloric value was 2540 per day The figures have been calculated from data from the Nederlandse Voedingsmiddelen Tabel (1958). The food consisted daily of 200 g. potatoes, 75 g. cauliflower de hydrated apples or carrots, 150 g. compote 15 g. bacon, 25 g. cheese or 1 hen's egg, 400 g. porridge, 175 g. low-protein bread, 60 g. natural butter 100 g. sugar 25 g. whipped cream, 10 g. jam. Further he

salt diet (figs. 86 87) the excretion of sulphur was not affected (fig. 85)

e) The excretion of calcium also increased during the administration of additional NaCl (fig 88).

In considering any of these balances it must be remembered that some after effect of NAPP may possibly have been involved

Balance tests in a patient aged 32 with chronic nephritis

CASE HISTORY – In 1946 an orthostatic albuminuria was observed in this previously healthy man. Soon afterward constant albuminuria and moderate hypertension could be demonstrated. In March 1947 a urea clearance of 50 / was found with a blood urea concentration of 352 mg. per litre.

Between 1946 and 1955 there were few disturbances. The blood pressure was 160/90 on the average. Apart from salt restriction no dietary limitations were imposed.

Toward the middle of 1955 the patient started to have complaints, especially headache vertigo and palpitations A urea clearance of approximately 10 / was found, with a blood urea concentration of 1620 mg per ml The blood pressure was 180/105 A salt free low protein diet was prescribed, with considerable restriction of meat, eggs fish, dairy products (with the exception of cream) and beans but with bread permitted ad libitum After an accident in which the patient broke both wrists the uraemia increased to a considerable degree, which caused the physician in charge to become suspicious that the dietary prescriptions were not being followed strictly enough The patient whose appetite was good, consumed approximately 450 g. bread per day containing 8 / protein It was therefore clear that he was getting too much protein, the more so as the breakdown of tissue had increased owing to the fractures.

In January 1956 the patient came under my care. He complained of great fatigue nausea and headache. From time to time he suffered from itching. The lower extremities and the face showed a swelling that varied every day The general nutritional condition was fairly good. The skin was of a yellowish pale colour The legs showed a mild oedema

Cardio-vascular abnormalities were not encountered

The only abnormality of the urine was a slight albuminuria.

The sodium, potassium, chloride and calcium levels and the CO combining power of the blood were normal, as was the level of the serum protein fractions. There was a normochromic anaemia the haemoglobin concentration was 10 4 g. per 100 ml.

The urea clearance was

$$\frac{5020}{1865} \times \sqrt{\frac{1482}{1440}} = 2.7 \text{ ml./min. or } 5\ 0\ \%$$

From this formula it can be concluded that 7.2 g. urea was excreted per 24 hours. Taking into account the excessive blood urea concentration as well, a diet was prescribed that contained 18 g protein and yielded 2500 calories. One week after this diet was started the urea clearance was

$$\frac{3990}{1448} \times \sqrt{\frac{1238}{1440}} = 2.6 \text{ ml /min or } 4\ 8\ \%$$

It is noteworthy that the urea level of the blood had decreased in one week from 1865 to 1448 mg. per litre in spite of a slight decline in renal function

A few days later the patient was put on a more standardized diet with which over a period of 45 days the same food was given every 3rd day and the urinary and faecal excretions of the protein catabolites were examined. Immediately after

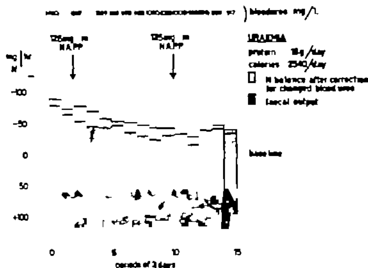


Fig. 91 Exp. XV Effect of NAPP on the N balance in patient with chronic uraemia during the administration of diet of adequate caloric value containing 18 g. protein per day
The renal excretion is plotted as block consisting of two white and one hatched areas. The upper white areas represent the corrections in the balance based on the changes in the blood urea level. The hatched areas represent the protein catabolism.

received 1500 ml. fluid in the form of tea, coffee, orange juice or apple juice. The porridge contained per 100 ml. 10 g. natural butter 10 g. sugar and 5 g. tapioca flour The butter and sugar used in this porridge were given in addition to the quantities mentioned above. The low-protein bread was prepared from wheat starch and locust bean flour (BOTIDMA, 1956). N sod um chloride was added to the diet. The food was the same every 3rd day and for this reason the urine was examined in 72 hour specimens.

Three times per day the patient was given a powder which contained 1 g. calcium carbonate and 200 mg. magnesium oxide. This counteracted the shortage of calcium n the food, and the mixture is also efficaceous against acidosis. Also the excess calcium caused a considerable f ecal excretion of phosphorus.

The aqueous vitamin mixture that has been previously described was added to the diet in a dose of 0.5 ml three times per day Evident vitamin deficiencies have never been observed with these low-protein diets, although several substances from the vitamin-B group are present in smaller quantities than are generally recommended.

Six days after the beginning of the experiment 125 mg. NAPP was injected intramuscularly and such an injection was given again on the 30th day

At the beginning of the experiment, the urea clearance was 4 6 / and the blood urea concentration amounted to 1420 mg./l.

The results are listed in table XVI and shown graphically in fig. 90

(1) In fig. 91 the nitrogen balance is shown only after correction for changes in blood urea concentration we obtain a clear impression of the degree of protein

URAEMIA
PROTEIN 18 gm/day
CALORIES 2540/day

Fig. 90 Exp XV 3... *Chronic uraemia*
Effect of nor-androstenolone phenylpropionate (Durabolin, NAPP) on the excretion of protein catabol es in a patient with chronic uraemia during the administration of a diet of adequate caloric lue containing 18 g. protein per day

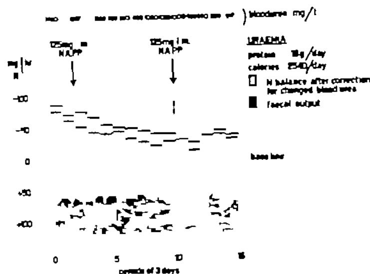


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Three times per day the patient was given a powder which contained 1 g. calcium carbonate and 700 mg. magnesium oxide. This counteracted the shortage of calcium in the food, and the mixture is also efficaceous against acidosis. Also the excess calcium caused a considerable faecal excretion of phosphorus.

The aqueous vitamin mixture that has been previously described was added to the diet in a dose of 0.5 ml. three times per day Evident vitamin deficiencies have never been observed with these low-protein diets, although several substances from the vitamin-B group are present in smaller quantities than are generally recommended.

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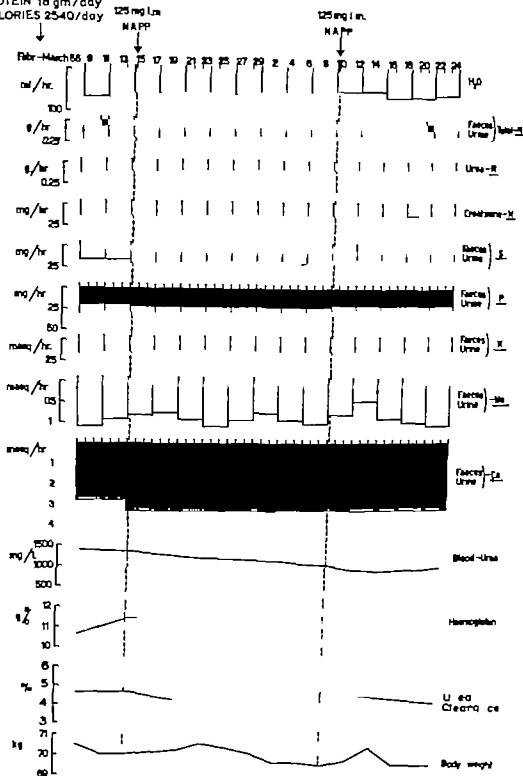


Fig. 90 Exp. XV ♂ 32. *Chronic uraemia*.
Effect of nor-androstenolone phenylpropionat (Durabolin, NAPP) on the excretion of protein catabolites in a patient with chronic uraemia during the administration of diet of adequate caloric value containing 18 g. protein per day

this figure was approximately 11 7 g. That means that the faecal loss of nitrogen was more than 50 / of the intake. Thus the net intake with a diet containing 20 g. protein is very low in uraemic patients.

(5) The large quantity of calcium carbonate in the diet led to a considerable faecal calcium excretion (fig. 90). Under the influence of the excess of calcium (PETERS and VAN SLYKE, 1946), high faecal excretion of phosphorus was also achieved (fig. 93). 511 mg. phosphorus was excreted per 24 hours, whereas the faecal excretion of phosphorus in the healthy man from the 2nd experiment amounted to 156 mg. It is not known what retained substances cause the signs and symptoms of uraemia. It is probable, however that the additional excretion of phosphorus is of therapeutic significance.

(6) The faecal excretion of potassium amounted to approximately 5 m.eq. per 24 hours, whereas in the second experiment 11 m.eq per day had been found (fig. 94). SMITH (1951) mentions the possibility of an increased tubular excretion of potassium in uraemia, as the result of which, even with severe renal insufficiency hyperpotassaemia does not of necessity occur It is probable, that the lack of a large faecal potassium excretion in uraemia is also due to this.

(7) It appears from fig. 90 that after the injections of NAPP a slight retention of sodium could be perceived, which amounted maximally to 0.5 m.eq per hour or 12 m.eq per 24 hours, corresponding to less than 0.5 m.eq. sodium per litre extracellular fluid.

XVI Between November 29 and December 31 1956, inclusive, the diet of the same

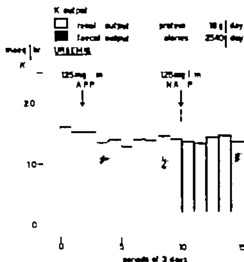


Fig 94 EXP XV Effect of NAPP on the K excretion in patient with chronic uraemia during the administration of diet of adequate caloric value containing 18 g. protein per day

patient was once more standardized (SIXTEENTH EXPERIMENT). The diet contained 15 g. protein 379 g. carbohydrate and 130 g. fat. The other conditions were the same as in the previous test, the caloric value once more amounting to 2540 per day

At the beginning of the experiment the urea clearance was 4.3 % and the blood urea concentration 1460 mg./l.

One injection of 125 mg. NAPP was given, 18 days after the beginning of the experiment.

The results are listed in table XVII and presented diagrammatically in fig. 95.

(1) It appears from fig. 96 that also in this case the protein catabolism decreased in the course of the experiment. In the last period before the administration of NAPP the catabolism of protein exceeded the production by some 5½ g. per day When we compare this slight loss of protein with the amount of protein in one litre of blood (approximately 200 g.), the significance of

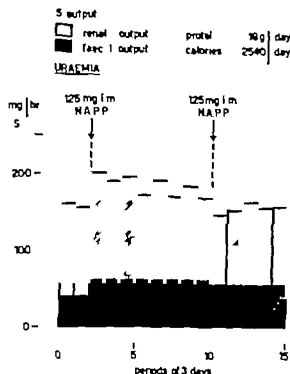


Fig. 92 – Exp XV – Effect of NAPP on the S excretion in a patient with chronic uraemia during the administration f a diet of adequate calori value containing 18 g. protein per day

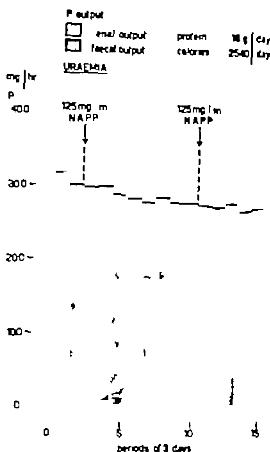


Fig. 93 Exp XV M rked faecal P excretion caused by oral administration of C in patient ith chronic uraemia during administration of diet of adequate caloric alue containing 18 g protein per day

catabolism. In the course of the experiment the degree of protein catabolism decreased gradually in the beginning the catabolism exceeded the production by about 12 g. protein, later on this figure was approximately 5 g.

(2) The influence of NAPP on the nitrogen excretion is difficult to assess from the figures. The impression is gained that some degree of protein synthesis has been brought about but owing to the disturbing influence of the possible spontaneous alterations we cannot calculate what degree.

(3) The excretion of sulphur is shown in fig. 92. For the assessment of the protein metabolism this figure is of less significance, because no determinations of sulphur in the blood have been carried out.

(4) The faecal excretion of nitrogen and sulphur was more than during the 2nd experiment in which approximately the same low protein diet was prescribed The faeces of the healthy man contained 840 mg. nitrogen and 78 mg. sulphur per day whereas the patient with uraemia excreted 1864 mg. nitrogen and 134 mg. sulphur per day with the faeces. These figures are not wholly comparable because the diets were not completely identical but nevertheless they give some impression of the significance of the faecal excretion in uraemia In the healthy man the excretion corresponded to the catabolites of approximately 5 3 g. protein and in the patient with uraemia

this figure was approximately 11 7 g. That means that the faecal loss of nitrogen was more than 50 / of the intake. Thus the 'net intake with a diet containing 20 g. protein is very low in uraemic patients.

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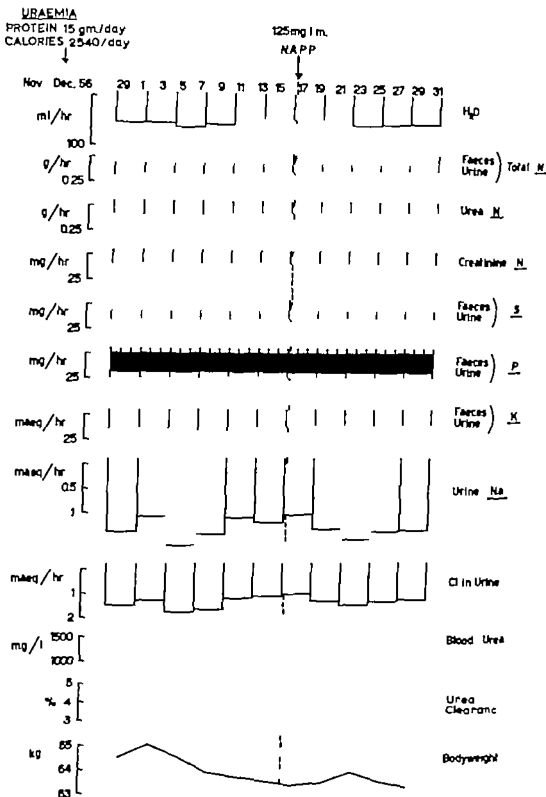


Fig. 95 – Exp. XVI ♂ 33. *Chronic uraemia.*
Effect of nor-androstenolone phenylpropionate (Durabolin, NAPP) on the excretion of protein catabolites in a patient with chronic uraemia during the administration of diet of adequate caloric value *containing 15 g.* protein per day. Same subject as in fig. 90.

a blood transfusion in chronic uraemia is obvious. It has repeatedly been demonstrated that the excretion of nitrogen does not increase significantly after blood is administered to a patient with chronic nephritis (BORST 1948).

(2) Like in the previous experiment, it could not be clearly determined whether the protein metabolism at the end of the experiment was still affected by NAPP

(3) It appears from figs. 96, 97 and 98 that the faecal excretions of nitrogen, sulphur and phosphorus were considerable, whereas the quantity of potassium in the faeces was again lower than in the healthy man in the second experiment (fig. 99). The faecal excretion of nitrogen, sulphur and phosphorus showed no appreciable decrease in the course of the experiment, so that a direct correlation with the quantity of urea in the blood probably did not exist.

(4) From fig. 95 it appears that during the first 3-day period after administration of NAPP there was a slight retention of sodium and chloride to be observed.

III. Conclusions from combinations of observations

1 *Determination of protein catabolites in faeces average faecal excretion of nitrogen per 24 hours*

It is often difficult to decide to what experimental period a given defaecation must be considered to belong. In view of the methods selected, for the determination of sulphur and phosphorus, it was not possible to mark the faeces with carmine red or carbo medicinalis, and two beads swallowed at the same time were sometimes recovered from different defaecations.

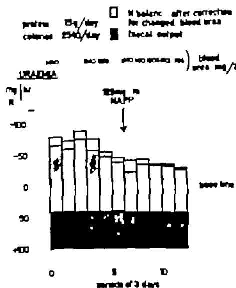


Fig 96 Exp XVI Effect of NAPP on the N balance in patient with chronic uraemia during the administration of diet of adequate caloric value containing 15 g. protein per day
The renal excretion is plotted as block consisting of two white and one hatched areas. The upper white areas represent the corrections in the balance based on the changes in the blood urea level. The hatched areas represent the protein catabolism.

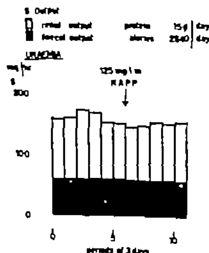


Fig 97 Exp XVI Effect of NAPP on the S excretion in patient with chronic uraemia during the administration of diet of adequate caloric value containing 15 g. protein per day

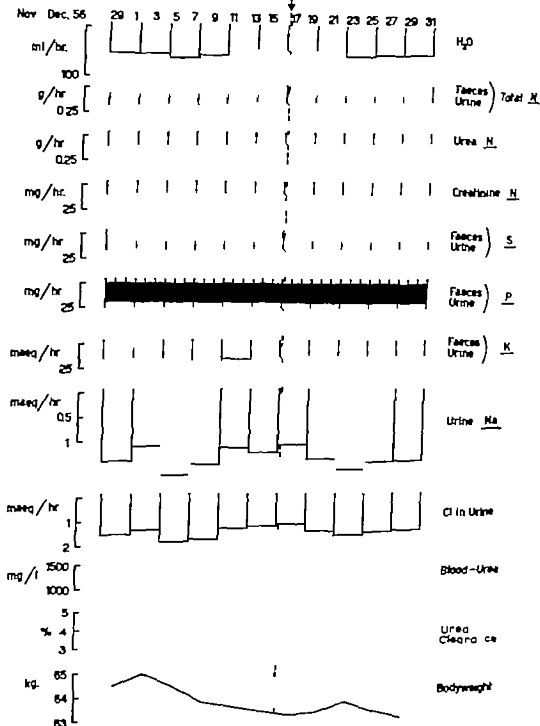


Fig. 95 – Exp XVI ♂ 33. *Chronic uraemia.*
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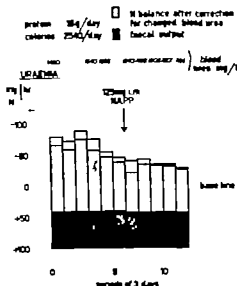


Fig 96 Exp XVI Effect of NAPP on the N balance in patient with chronic uraemia during the administration of diet of adequate caloric value containing 15 g. protein per day
The renal excretion is plotted as block consisting of two white and one hatched areas. The upper white areas represent the corrections in the balance based on the changes in the blood urea level. The hatched areas represent the protein catabolism.

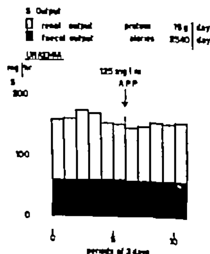


Fig 97 Exp XVI Effect of NAPP on the S excretion in patient with chronic uraemia during the administration of diet of adequate caloric value containing 15 g. protein per day

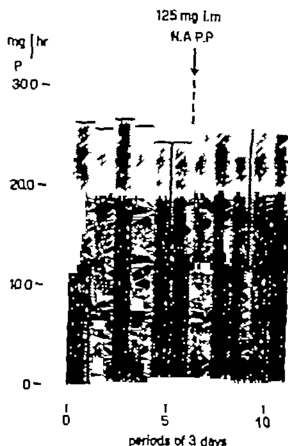


Fig. 98 Exp XVI Marked faecal P excretion caused by oral administration of Ca in patient with chronic uraemia during administration of diet of adequate caloric value containing 15 g protein per day

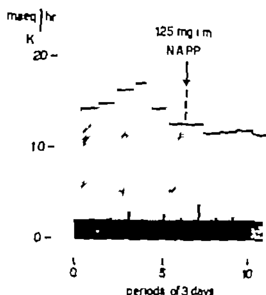


Fig. 99 Exp XVI – Effect of NAPP on the K excretion in patient with chronic uraemia during the administration of a diet of adequate caloric value containing 15 g. protein per day.

After important changes in the composition of the diet the appearance of the faeces as a rule was sufficiently different to do without marking However the assessment was often impossible when a slight alteration of the diet was concerned and also it was impossible to determine with accuracy whether a defaecation belonged to a period before or after the administration of an anabolic steroid For these reasons in a number of experiments it was necessary to determine the average of the faecal excretions over the whole duration of the test. The errors which may be made in this way are great when the excretion with the faeces constitutes a large percentage of the total, as is the case in persons with a low protein catabolism.

In one patient with diminished renal function the faecal loss of nitrogen, sulphur and phosphorus was increased not only relatively but also absolutely The excretion of phosphorus with the faeces in healthy persons also often constitutes a large percentage of the total excretion, particularly when the diet contains much calcium.

In some cases it may be necessary to

TABLE XVIII

THE AVERAGE FAECAL EXCRETION OF NITROGEN PER 24 HOURS (IN GRAMMES)

exp	*diet*			*particulars*	*faecal excretion per 24 hours in grammes*
	calories		*protein*		
	per day	*per kg B W*	*g p day*		
I	2400	28.0	0	sugar exclusively	0.24
II	3190	38.0	22½	varied diet	0.84
	3190	38.0	137	id.	0.94
III	500	6.0	20.4	low-cellulose	0.29
	1000	13.3	20.8	id.	0.38
	1500	20.0	21.1	id.	0.43
	2000	27.5	21.5	id.	0.43
	2500	34.3	21.8	id.	0.43
	3000	40.8	22.2	id.	0.36
	3500	47.0	22.5	id.	0.54
IV	2250	40.0	22	id.	0.84
V	2980	38.9	80	varied diet	0.73
	2530	38.9	80	id.	0.71
	1600	22.2	0	sugar exclusively	0.35
	1600	25.4	0	id.	0.23
	400	5.3	0	id.	0.43
	400	6.7	0	id.	0.18
VI	2000	28.2	96	milk protein, butter sugar	0.96
VII	2144	30.6	80	id.	0.72
VIII	2400	33.8	80	id.	0.55
IX	2400	35.0	20	id.	0.60
X	2370	38.2	80	id.	0.48
XI	2100	38.2	20	id.	0.46
XII	2690	38.0	80	id.	0.36
XIII	2450	38.0	20	id.	0.58
XIV	2450	35.5	20	id.	0.43
XV	2540	36.0	18	patient with uraemia	1.44
XVI	2540	40.0	15	id.	1.20

administer laxatives and enemas at the end of each experimental period.

It is not correct to estimate the faecal excretion of protein metabolites as a percentage of the quantities ingested with the food. Fig. 15 gives an impression of the errors that can be made if it is assumed that there exists a fixed proportionality between the faecal excretion of nitrogen and the quantity of protein in the food. The error can be limited considerably if it is assumed that, regardless of the food-intake, the same quantity of protein catabolites is excreted per day. The quantity of nitrogen that is excreted with the faeces per 24 hours is usually estimated as 1.3 g. Table XVIII presents a review of the average daily faecal excretions of nitrogen during the 16 experiments. It is clear that in our experiments only in the patient

with uraemia the faecal nitrogen exceeded 1 g. per day

2. *Saving of protein by carbohydrates and fats the nitrogen balance during administration of low-protein diets*

A number of the experiments reported have supplied data concerning the significance of the caloric value of the food for protein catabolism. As a rule, protein-free diets or very low protein diets were concerned as the purpose of the investigations was to study the optimal composition of the food in patients suffering from chronic uraemia or from acute anuria. In acute anuria the sole aim is a protein catabolism as low as possible, as measured from the variable part of the protein metabolites in the urine. In chronic uraemia we must attempt to obtain nitrogen equilibrium with a protein catabolism that is as low as possible which requires a compromise with these diets both the nitrogen balance and the amount of protein catabolites are of significance

(a) In two experiments, the rôle of various quantities of sugar in protein catabolism with protein free diets has been studied In the IVth experiment, a healthy woman had consumed only 22 g. protein per 24 hours for 81 days, during which period the body weight decreased from 56.2 to 53 6 kg. From figure 23 we can calculate that in all, approximately 690 g. protein was lost, plus the extra loss of protein during 4 menstrual periods and less approximately 17 g. which corresponds to the decrease of the urea concentration in the body fluids during the observation period As the result of this calorically almost adequate, low-protein diet, the protein catabolism, calculated on the basis of the so-called variable nitrogen with the urine, urea and ammonia, had finally become very low corresponding to approximately 20 g. protein per 24 hours. When immediately after this diet another diet was given which consisted of 100 g. sugar only the protein catabolism decreased still further on the 4th day of this diet the variable nitrogen in the urine amounted to 2.4 g. corresponding to 15 g. protein. After this diet had been administered for 6 days, the daily quantity of sugar was increased from 100 to 300 g. As the result of this change the urine on the 4th day of the higher calorie diet contained 1 7 g. variable nitrogen, corresponding to 11 6 g. protein Expressed in percentages (29 %) the protein-saving was therefore considerable, but quantitatively the saving effect of the extra calories was of little significance The 4th day of the diets was chosen in order to make possible a correct comparison with the next experiment.

In the Vth experiment the rôle of sugar in the protein metabolism in a normal nutritional condition was studied in two healthy men For the first 5 days both received a calorically adequate diet containing 80 g. protein dayly after which one of them was put on 100 g. sugar and the other on 400 g. sugar per day exclusively After a non-standardized interval of normal nutrition both were put on the same diet containing 80 g. protein, after which the same quantities of sugar were given again, but in the opposite order The excretion of variable nitrogen with the urine during the periods of the calorically complete diets, containing *80 g. protein, amounted* to approximately 12 g. per 24 hours, de-

riving from 75 g. protein. On the 4th day of the diet of 100 g. sugar these excretions had decreased to 10.5 and 8 6 g. nitrogen respectively corresponding to 65.6 and 53.8 g. protein respectively i.e. considerably more than in the previous experiment in which on the same day of the experiment the variable catabolites of only 15 g. protein were excreted. On the 4th day of the administration of 400 g. sugar the quantities of variable nitrogen in the urine had decreased to 5.2 and 5.8 g. respectively corresponding to 32.5 and 36.3 g. protein respectively

In other words, in the Vth experiment on the days selected for comparison a protein-saving was obtained by extra sugar of 33 1 and 17.5 g. respectively whereas in the previous experiment this quantity was only 3 4 g. In percentages therefore the gain in the Vth experiment was 50 / and 33 / respectively and 29 / in the IVth experiment. The absolute value of the additional protein-saving obtained by increasing the quantity of sugar to over 100 g. per 24 hours was therefore greater in the men who had previously been on a normal diet than in the woman who for a long time had been on a low-protein diet. In percentages, however there was a similarity of the degree of additional nitrogen saving in the 2 experiments.

In table XIX (p. 120) a number of comparative data are listed, including the total nitrogen excretions. All figures have been calculated over the first 4 days of a sugar diet and over the 4th 24-hour period in which this diet was administered. A correction has been applied for the alterations of the blood urea concentration. A determination was also made of how much the blood urea concentration would have increased after the first 4 days and during the 4th 24-hour period, if there had been an anuria.

From these observations it appears that there are important quantitative differences of the degrees of additional protein-saving after increase of the quantity of sugar in the diet. Following an optimal nutritional condition the extra sugar played a much greater part than in the other experiment, where the protein catabolism was already low when the sugar diet was started, because the subject had been on a low-protein diet for a considerable length of time previously The differences between the percentages of the decrease of the protein catabolism under the influence of extra sugar were much less, however Although the fifth experiment is not completely comparable with the fourth (the quantities of sugar after the increase of the daily diet were not the same, and the influence of bed rest was absent in the IVth experiment) there yet are so many points of similarity between the arrangement of the experiments, that importance can be attached to the quantitative differences that have been observed.

Earlier on a description was given of an experiment carried out by GAMBLE (1946), who in a healthy man who had been fasting for 6 days, observed an important protein-saving when 100 g. of glucose were administered, whereas increase of this quantity of glucose to 200 g. had practically no further effect on the rate of protein catabolism. Figs. 39 40 and 43 give a comparative impression of the saving of protein by extra sugar in the Vth experiment and in Gamble's test. We refer once more to these figures, because this single

with uraemia the faecal nitrogen exceeded 1 g. per day

2. Saving of protein by carbohydrates and fats the nitrogen balance during administration of low protein diets

A number of the experiments reported have supplied data concerning the significance of the caloric value of the food for protein catabolism. As a rule, protein-free diets or very low-protein diets were concerned as the purpose of the investigations was to study the optimal composition of the food in patients suffering from chronic uraemia or from acute anuria. In acute anuria the sole aim is a protein catabolism as low as possible, as measured from the variable part of the protein metabolites in the urine. In chronic uraemia we must attempt to obtain nitrogen equilibrium with a protein catabolism that is as low as possible, which requires a compromise with these diets both the nitrogen balance and the amount of protein catabolites are of significance

(a) In two experiments, the rôle of various quantities of sugar in protein catabolism with protein-free diets has been studied. In the IVth experiment, a healthy woman had consumed only 22 g. protein per 24 hours for 81 days, during which period the body-weight decreased from 56.2 to 53 6 kg. From figure 23 we can calculate that in all approximately 690 g. protein was lost, plus the extra loss of protein during 4 menstrual periods and less approximately 17 g. which corresponds to the decrease of the urea concentration in the body fluids during the observation period. As the result of this calorically almost adequate, low-protein diet, the protein catabolism, calculated on the basis of the so-called variable nitrogen with the urine urea and ammonia, had finally become very low corresponding to approximately 20 g. protein per 24 hours. When immediately after this diet another diet was given, which consisted of 100 g. sugar only the protein catabolism decreased still further on the 4th day of this diet the variable nitrogen in the urine amounted to 2.4 g. corresponding to 15 g. protein. After this diet had been administered for 6 days, the daily quantity of sugar was increased from 100 to 300 g. As the result of this change the urine on the 4th day of the higher calorie diet contained 1 7 g. variable nitrogen corresponding to 11 6 g protein Expressed in percentages (29 %) the protein-saving was therefore considerable, but quantitatively the saving effect of the extra calories was of little significance. The 4th day of the diets was chosen in order to make possible a correct comparison with the next experiment

In the Vth experiment the rôle of sugar in the protein metabolism in a normal nutritional condition was studied in two healthy men For the first 5 days both received a calorically adequate diet containing 80 g. protein dayly after which one of them was put on 100 g. sugar and the other on 400 g. sugar per day exclusively After a non-standardized interval of normal nutrition both were put on the same diet containing 80 g. protein, after which the same quantities of sugar were given again, but in the opposite order The excretion of variable nitrogen with the urine during the periods of the calorically complete diets, containing 80 g. protein, amounted to approximately 12 g. per 24 hours, de-

observation of Gamble has been taken by MERRILL (1955 1960) as the basis of the dietetic treatment of acute anuria. However Gamble had taken into account the excretion of nitrogen in urine and faeces, but not the alterations of the quantity of nitrogen-containing protein catabolites in the blood. Moreover patients with acute anuria are frequently persons in a normal nutritional condition, whose production of urine is arrested suddenly so that we may expect a quantitatively much greater protein-sparing influence of extra sugar (comparable with the Vth experiment) than in Gamble's experiment in which the subject had previously been fasting for 6 days with a loss of protein, so that there is a similarity with the conditions of the IVth experiment, in which there had been a negative nitrogen balance for 81 days, when the sugar diets were compared.

For these reasons it is fundamentally incorrect to use Gamble's observation as an argument in the discussion of the treatment of acute anuria. The quantitative significance of the protein-saving of additional administration of sugar beyond 100 g. per day is difficult to predict for each individual case of acute anuria. It depends on a number of factors, of which a very important one is the degree of protein catabolism prior to the arrest of the urine production. In both the IVth and the Vth experiment the percentage of additional decrease of the urea production, due to increase of the quantity of sugar to more than 100 g. per day was of significance. One does not know how great will be the saving influence in case of enhanced protein catabolism due to damage of tissues. When percentually this is of the same order as in the experiments reported, the difference in effect between 100 and 300 or 400 g. sugar is quantitatively of great importance

The conclusions concerning the great significance of additional sugar above 100 g. for the degree of protein catabolism are supported by the observations of the differences between the excretions of sulphur phosphorus and potassium with diets of 100 g. sugar and calorically richer sugar diets. This point is well illustrated by figs. 33 and 34 and by the figures from table XXI.

It follows from the above that the diet in acute anuria must always supply more calories than that provided by 100 g. sugar The experiments do not answer the question what is the optimal caloric value of the diet.

As far as the data from the review of the literature show there are no theoretical objections against making fat a part of the diet. However in order to prevent ketosis, which increases the protein catabolism, it is necessary for the diet to contain at least 100 g. carbohydrates.

(b) In some other experiments attention has been paid to the relationship between the caloric value of the diet and the protein balance. This correlation as appears from the results of the IIIrd experiment, in which some 20 g. protein per day were consumed for 156 days, is once more illustrated in fig. 100. By increasing the caloric value from 500 to 1000 a protein-saving of 4.5 g. per day was obtained. The next 1000 calories decreased the protein catabolism by 6.6 g., i.e. 3.3 g. per 500 calories. By subsequently increasing the caloric value of the diet by 500 calories per 24 hours at a time, a protein-saving

TABLE XIX

experiment diet	*IV*		*V subject A*		*V subject B*	
	100 g sugar after low-prot diet	*300 g sugar after low-prot diet*	*100 g sugar after optimal diet*	*400 g sugar after optimal diet*	*100 g sugar after optimal diet*	*400 g sugar after optimal diet*
total N-excretion 4 days	14.3 g	11 7 g	46.2 g	27 9 g	39 0 g.	29 4 g
total N-excretion 4th day	3 4 g	2.8 g	11 8 g	6.4 g	9 7 g	6.9 g
variable N in urine 4 days	10 1 g	7 4 g	40.7 g	23 7 g	34.2 g	25 0 g
variable N in urine 4th day	2.4 g	1 7 g	10.5 g	5.2 g	8 6 g	5 8 g
/ decrease variable N 4 days		27 /		42 /		27 /
/ decrease variable N 4th day		29 /		50 /		33 /
calculated increase urea /l. blood 4 days [1]	508 mg	379 mg	1467 mg	854 mg	1471 mg	1075 mg
calculated increase urea /l blood 4th day [1]	121 mg	87 mg	378 mg	187 mg	370 mg.	249 mg

[1]) Provided no urine would have been produced.

observation of Gamble has been taken by MERRILL (1955, 1960) as the basis of the dietetic treatment of acute anuria. However Gamble had taken into account the excretion of nitrogen in urine and faeces, but not the alterations of the quantity of nitrogen-containing protein catabolites in the blood. Moreover patients with acute anuria are frequently persons in a normal nutritional condition whose production of urine is arrested suddenly, so that we may expect a quantitatively much greater protein-sparing influence of extra sugar (comparable with the Vth experiment) than in Gamble's experiment in which the subject had previously been fasting for 6 days with a loss of protein, so that there is a similarity with the conditions of the IVth experiment, in which there had been a negative nitrogen balance for 81 days, when the sugar diets were compared.

For these reasons it is fundamentally incorrect to use Gamble's observation as an argument in the discussion of the treatment of acute anuria. The quantitative significance of the protein-saving of additional administration of sugar beyond 100 g. per day is difficult to predict for each individual case of acute anuria. It depends on a number of factors, of which a very important one is the degree of protein catabolism prior to the arrest of the urine production. In both the IVth and the Vth experiment the percentage of additional decrease of the urea production, due to increase of the quantity of sugar to more than 100 g. per day, was of significance. One does not know how great will be the saving influence in case of enhanced protein catabolism due to damage of tissues. When percentually this is of the same order as in the experiments reported, the difference in effect between 100 and 300 or 400 g. sugar is quantitatively of great importance.

The conclusions concerning the great significance of additional sugar above 100 g. for the degree of protein catabolism are supported by the observations of the differences between the excretions of sulphur, phosphorus and potassium with diets of 100 g. sugar and calorically richer sugar diets. This point is well illustrated by figs. 33 and 34 and by the figures from table XXI.

It follows from the above that the diet in acute anuria must always supply more calories than that provided by 100 g. sugar. The experiments do not answer the question what is the optimal caloric value of the diet.

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variable N in urine 4 days	10.1 g	7 4 g	40.7 g	23 7 g	34.2 g	25.0 g
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(b) In some other experiments attention has been paid to the relationship between the caloric value of the diet and the protein balance. This correlation as appears from the results of the IIIrd experiment, in which some 20 g. protein per day were consumed for 156 days, is once more illustrated in fig. 100. By increasing the caloric value from 500 to 1000 a protein saving of 4.5 g. per day was obtained. The next 1000 calories decreased the protein catabolism by 6.6 g., d.e. 3.3 g. per 500 calories. By subsequently increasing the caloric value of the diet by 500 calories per 24 hours at a time, a protein-saving

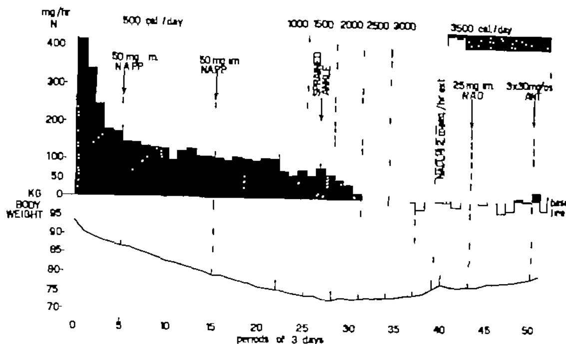


Fig. 100 – Exp III – ♂ 48. The influence of the caloric intake on the N balance during the administration of diets containing from 20.4 to 22.5 g. per day

was obtained of 3.0, 1.2 and 0.7 g. per day respectively. Consequently we obtain a curve with a tendency to an asymptotic course, of which the phase with the approximately straight course, at the level of nitrogen equilibrium, begins only with a caloric value of the diet which is some 500 calories more than are needed to keep the body weight stable. Under the experimental conditions selected, with a little *more than 20 g. protein in the diet, nitrogen* equilibrium could only be obtained when the food yielded 35 calories per kg. body-weight, while the body weight increased already after the caloric value had been raised to 27½ per kg. In evaluating the nitrogen excretions we must take into account a 0.37 g. increase of the protein in the diet for each increase of the caloric value by 500.

It appears from fig. 100 that a caloric value, above which practically no further improvement of the nitrogen balance can be obtained, does indeed exist; however this caloric value is more than the value necessary for the maintenance of the body-weight at the same level.

Consequently it proved impossible in other experiments, to obtain nitrogen equilibrium with diets which had a just sufficient caloric value and which contained approximately 20 g. protein.

(1) In the IInd experiment, a calorically *adequate diet was given which contained* 22½ g. protein. After 30 days the catabolism of protein still exceeded the production by 5.1 g. per day. Also 60 days later the nitrogen balance was still slightly negative.

(2) In the IVth experiment a healthy woman received a diet which yielded 2250 calories and contained 22 g. protein. After 81 days the nitrogen balance was still negative, indicating a loss of approximately 7 g. protein per day, but the decrease of the body-weight revealed that the caloric value of the diet was not quite sufficient.

(3) In various experiments of shorter duration healthy men during bed rest received a diet which calorically was wholly or practically sufficient and which contained 20 g. protein. The nitrogen balances in all cases remained distinctly negative. However it is known from the literature, as well that bed rest causes a considerable catabolism of protein.

(4) In the XVth and XVIth experiments, a patient with severe uraemia received a calorically wholly sufficient diet containing 18 and 15 g. protein respectively After 45 and 33 days, respectively the nitrogen balances were still negative 4.8 and 5 1 g. protein respectively were lost per day It should be noted that the negative balance was largely the result of the loss of 1 3 g nitrogen, equivalent with 8 1 g. protein, with the faeces.

These observations show that the prescription of a diet containing 20 g. protein per day or even less, to a patient with chronic uraemia must be regarded as an evil which must be carefully weighed against the necessity Very low-protein diets are of importance especially when a high blood-urea level is associated with complaints of nausea, vomiting and anorexia so that the caloric value of the food intake may become insufficient for the desired protein-anabolic effect.

The fact that the first three experiments started after a period of normal nutrition and have been carried out in the same man makes possible a more accurate comparison especially of the initial 18-day periods which in none of the three experiments were disturbed by a change of diet or administration of an anabolic steroid In the first experiment the diet consisted exclusively of 600 g sugar per day In the second experiment, a calorically sufficient diet was given which contained 22½ g. protein the diet in the third experiment yielded 500 calories and contained 20.4 g. protein In table XX (p 124) a number of data are shown attention is given to the total excretion of nitrogen in urine and faeces and to the quantity of variable nitrogen in the urine (in the Ist and IIIrd experiment, urea and ammonia, in the IInd, total nitrogen in the urine with substraction of creatinine) and the calculated increase of the urea concentration of the blood when anuria would have been present. These data have been calculated for a period of 18 days and also for the 19th experimental day Consequently with a protein-free diet yielding 2400 calories, the work of the kidney in excreting the variable nitrogen originating from protein catabolism was about the same as with a diet containing 22½ g. protein and yielding 3190 calories while the excretion of this nitrogen was twice as much with a diet which yielded 500 calories and contained 20 4 g. protein

(c) With a diet containing sugar exclusively it was found that the nitrogen excretion was reduced by administering the sugar in several small portions (figs. 3 4 5, 6). Although no important differences have been encountered it is nevertheless to be recommended that, e g with parenteral feeding the infusions containing carbohydrates be given at reasonably brief intervals.

(d) The excretion of nitrogen proved to be significantly more with a diet without carbohydrates than with a diet in which part of the fat had been replaced isocalorically by sugar (figs. 52 and 53). This confirms the statements made previously

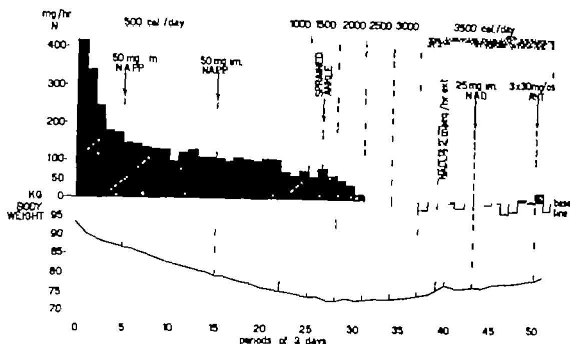


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(3) In various experiments of shorter duration healthy men during bed rest received a diet which calorically was wholly or practically sufficient and which contained 20 g. protein. The nitrogen balances in all cases remained distinctly negative. However it is known from the literature, as well, that bed rest causes a considerable catabolism of protein.

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These observations show that the prescription of a diet containing 20 g. protein per day or even less, to a patient with chronic uraemia must be regarded as an evil, which must be carefully weighed against the necessity. Very low-protein diets are of importance especially when a high blood-urea level is associated with complaints of nausea, vomiting and anorexia, so that the caloric value of the food intake may become insufficient for the desired protein-anabolic effect.

The fact that the first three experiments started after a period of normal nutrition and have been carried out in the same man makes possible a more accurate comparison, especially of the initial 18-day periods which in none of the three experiments were disturbed by a change of diet or administration of an anabolic steroid. In the first experiment the diet consisted exclusively of 600 g. sugar per day. In the second experiment, a calorically sufficient diet was given which contained 22½ g. protein the diet in the third experiment yielded 500 calories and contained 20.4 g. protein. In table XX (p. 124) a number of data are shown attention is given to the total excretion of nitrogen in urine and faeces and to the quantity of variable nitrogen in the urine (in the Ist and IIIrd experiment, urea and ammonia, in the IInd, total nitrogen in the urine with substraction of creatinine) and the calculated increase of the urea concentration of the blood, when anuria would have been present. These data have been calculated for a period of 18 days and also for the 19th experimental day. Consequently with a protein-free diet yielding 2400 calories, the work of the kidney in excreting the variable nitrogen originating from protein catabolism was about the same as with a diet containing 22½ g. protein and yielding 3190 calories, while the excretion of this nitrogen was twice as much with a diet which yielded 500 calories and contained 20.4 g. protein.

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TABLE XX

diet	*cal 2400 protein. nil*	*cal 3190 protein. 22½ g*	*cal. 500 protein. 20 4 g*
total N excretion 18 days	94 g	102 g	167 g
total N excretion 19th day	3.5 g	4 4 g	6.7 g
variable N in urine 18 days	74 g	75 g	150 g
variable N in urine 19th day	2.4 g.	2.9 g	5.8 g
calculated increase urea/l. blood 18 days 1)	2350 mg.	2350 mg	4490 mg
calculated increase urea/l. blood 19th day 1)	77 mg	90 mg	180 mg

) Provided no urine would have been produced

concerning the necessity of administering carbohydrates in order to obtain optimal protein-saving. Still in the long run the body becomes somewhat better able to limit the catabolism of protein on a diet containing only protein and fat, as shown in the review of the literature The reduction of the nitrogen excretion after the 3rd day of a diet without carbohydrates points in the same direction (fig 52)

(e) The favourable reaction of carbohydrate on the protein synthesis depends in part on the interval between the ingestion of the protein and that of the carbohydrate the same is not true of fat. Figs. 48 and 49 show that the separate administration of protein and sugar with a fat-free diet causes loss of nitrogen figs 60 61 and 62 show that the same is true of a diet which also contains fat, while the separate ingestion of protein and fat causes no change of the excretion of the protein catabolites. This observation has no practical significance, because none of the prescriptions of protein saving diets such as the high-calorie, low-protein diet for uraemia are for meals containing

TABEL XXI

diet: calories p. day	diet: calories p. kg B W	diet: protein g/day	experiment nr	experiment day	particulars	loss of body protein g./day	excretion urea g./day	xcretion urine sulphur mg./day	excretion urine phosphorus mg./day	excretion urine potassium 1) m.eq./day
2400	28.0	0	I	19th	sugar only	20.9	3.84	254	144	9.6
3190	38.0	22.5	II	30th	varied diet	5.1	3.60	269	506	48
500	6.0	20.4	III	66th	varied diet	15.3	9.84	370	336	30
1000	13.3	20.8	III	9th	aft. 66 d. 500 cal.	12.0	8.26	370	341	28
2000	27.5	21.5	III	9th	experim. continued	3.8	4.66	269	295	27
2500	34.3	21.8	III	9th	experim. continued	1.4	4.12	269	295	28
3000	40.8	22.2	III	9th	experim. continued	1.1	4.37	278	295	24
3500	47.0	22.5	III	45th	experim. continued	1.5 (gain)	3.60	286	331	19
2250	40.0	22.0	IV	81st	varied diet	8.3	5.71	278	317	38
400	7.5	0	IV	6th	aft. 81 d. 22 g prot.	21.2	3.65	194	134	
1200	22.8	0	IV	6th	experim. continued	18.8	3.00	192	137	
400	5.3	0	V	4th	after normal diet	70.3	18.48	948	636	
1600	22.2	0	V	4th	Id.	41.9	9.41	456	576	
400	6.7	0	V	4th	Id.	61.1	15.22	586	648	
1600	25.4	0	V	4th	Id.	42.8	11.28	458	509	
2400	35.0	20.0	IX	5th	bed rest	18.5	7.68	324	286	
2400	35.0	20.0	IX	49th	Id.	9.0	4.80	187	274	
2100	38.2	20.0	XI	19th	Id.	8.7	6.24	264	175	
2450	38.0	20.0	XIII	20th	Id.	20.9	8.64	302	288	
2450	35.5	20.0	XIV	25th	Id.	14.7	6.34	302	307	
2540	36.0	18.0	XV	45th	chronic uraemia	4.8	[illegible].68	250	139	28
2540	40.0	15.0	XVI	33rd	Id.	5.1	2.35	230	144	24

1) Listed only when no potassium was added the diet.

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(e) The favourable reaction of carbohydrate on the protein synthesis depends in part on the interval between the ingestion of the protein and that of the carbohydrate the same is not true of fat. Figs. 48 and 49 show that the separate administration of protein and sugar with a fat free diet causes loss of nitrogen figs. 60 61 and 62 show that the same is true of a diet which also contains fat, while the separate ingestion of protein and fat causes no change of the excretion of the protein catabolites. This observation has no practical significance, because none of the prescriptions of protein-saving diets such as the high-calorie low-protein diet for uraemia are for meals containing

TABLE XXII

experim. nr	steroid	quantity in mg	diet: calor /day	diet: calor /kg	diet: protein g /day	diet: duration of diet	saving: total gain g prot	saving: period of saving days	saving: max. gain /day g protein	saving: on day after injection
XII	TP [1]	250	2450	38	20	9	26.4	5	9.0	4th
XI	TP	250	2690	38	80	9	99.0	6	17.1	2nd
II	TPP [2]	125	3190	38	22.5	60	18.8	12	2.3	4–6th
II	TPP	125	3190	38	137	21	63.0	6	10.5	4–6th
II	NAPP [3]	125	3190	38	22.5	30	33.1	12	3.5	4–6th
II	NAPP	125	3190	38	22.5	75	23.0	12	3.0	7–9th
II	NAPP	125	3190	38	137	39	57.6	6	9.6	4–6th
III	NAPP	50	400	6	20	15	?			
III	NAPP	50	500	6	20	45	0			
IV	NAPP	25	2250	40	20	18	4.5			
III	NAD [4]	25	3500	46	20	129	0			
IV	NAD	25	2250	41	20	45	0			
III	ANT [5]	3 × 30	3500	45	20	150	?			
IV	ANT	3 × 30	1700	24	0	12 [6]	0			
XV (uraemia)	NAPP	125	2540	36	18	many months	slight			
XVI (uraemia)	NAPP	125	2540	40	15	many months	slight			

1) Testosterone propionate (Neohombreol, Organon)
2) Testosterone phenylpropionate (Organon)
3) 19-Nor-androstenolone phenylpropionate (Durabolin, Organon)
4) 19-Nor-androstenolone decanoate
5) Ethylnor-testosterone (Nilevar, Searle)
6) After the administration of calorically almost adequate diet, containing 20 g protein for 81 days

protein and fat exclusively and it is known from the literature that there is no objection to part of the carbohydrates being replaced by fat.

(f) In table XXI (p 125) some data from various experiments are listed which give an impression of the protein catabolism with low-protein and protein free diets.

3 *Saving of protein by androgenic steroids*

(a) In the experiments described, the ad ministration of anabolic steroids was re stricted to a single or at the most a few injections. It goes without saying that it is more interesting to know the influence of a more protracted administration It can be deduced from the review of the literature that various investigators soon saw a so-called wearing off effect with continued administration of androgenic substances when the treatment was discon tinued however a few months later the same dose provoked the same degree of protein synthesis again It is to be ex pected, therefore that the greatest saving of nitrogen will be obtained when the steroids are administered periodically There are as yet no data in the literature concerning a wearing off effect of the newer weakly androgenic substances.

(b) It appears from the literature that the smaller the natural production of androgenic substances the greater is the protein-anabolic effect of androgenic substances. Possibly it is for this reason that a greater protein saving effect was obtained with 125 mg NAPP in a man aged 45 than in a man aged 20 both of whom were on a diet containing 20 g. protein (figs. 8 and 84)

(c) During the first day after the ad ministration of the rapidly acting TP the nitrogen excretion was somewhat increased, and the quantity of creatinine excreted was also greater (figs. 75 79 and 82) This increase of the nitrogen ex cretion on the day of the injection was demonstrated in experiments in dogs and rats, as has been reported earlier in the survey of the literature

It is possible that the enhanced ex cretions are attributable to a temporarily augmented function of the kidney However in the numerous experiments that have been cited, never before has any improvement of the renal function under the influence of anabolic steroids been demonstrated.

(d) Several of the present observations indicate that the influence of androgenic substances on the metabolism of protein is proportional to the amount of protein in the body

(1) During high-protein diets the administration of androgens was followed by a greater saving of protein than during diets containing little protein. This fact could be demonstrated after administration of MAD (figs. 67 and 71) and of TP (figs. 75 and 79)

(2) The protein saving effect of 50 mg NAPP during a diet of 500 calories containing some 20 g protein was inversely proportional to the length of time for which the subject had been on this diet (fig. 17)

(3) ANT caused a very slight nitrogen retention during a high-calorie low-protein diet in a man who had lost approximately 1 8 kg. protein and no nitrogen retention was seen after administration

(b) A gradual decrease during a diet of 3190 calories, which contained 22½ g. protein (fig. 14). In 80 days the excretion underwent a reduction of approximately 18 %.

(c) After replacement of the diet containing 22½ g. protein by an isocaloric diet containing 137 g. protein, the creatinine excretion increased during the first 3 days by more than 20 %, during the subsequent 9 days by more than 6 % and during the last period of 42 days by more than 10 %.

(d) A gradual decrease of almost 30 % was seen after the subject had for 66 days taken a diet, which contained 20.4 g. protein and yielded 500 calories.

(e) A gradual increase of approximately 5 % was observed with a diet, which contained 22½ g. protein and yielded 3500 calories. This diet was maintained for 45 days.

(f) The excretion underwent no changes when a calorically sufficient diet was given, containing 80 g. protein (fig. 82).

(g) A slow decrease of approximately 7 % was observed in the course of a period of 20 days, during which a calorically sufficient diet was given that contained 20 g. protein.

A number of these observations can be explained on the basis of decrease, stability or increase, respectively of the quantity of muscular tissue under conditions of protein loss, nitrogen equilibrium and positive nitrogen balance, respectively. The composition of the diet also plays a part, however: when the diet contains much meat (and consequently much creatinine) the excretion is greater. It sometimes appears that the excretion takes place at a slower rate. Possibly the gradual decrease in creatinine excretion during administration of a diet poor in creatinine must be partly explained by the presence of a depot of creatinine or more likely of a precursor of creatinine, which is gradually used up when less is ingested with the food. Therefore, the alterations of the creatinine excretion after changes of the diet may not be regarded as a quantitatively completely reliable indication of alterations of the quantity of muscular tissue.

The higher creatine content of a diet rich in meat is probably of no significance with regard to the observed sudden increase of the excretion of creatinine. Rose, Ellis and Helming (1928) administered 1 g. creatine per day to healthy adult subjects, and it lasted 14 days before this treatment led to an increased excretion of creatinine. This increased excretion then continued for some time after the administration of creatine had been discontinued. Similar observations have been described by Berglund (1922), Benedict and Osterberg (1923), Hahn and Meyer (1923), Chanutin (1926) and Hyde (1942). In some cases no increased excretion was observed after a single administration of 4 g. creatine.

(h) During the first 24-hour period after administration of 250 mg. TP with calorically complete diets, the excretion of creatinine increased distinctly. This increase was greater when the diet was rich in protein than with a diet containing 20 g. protein (fig. 82). The excretion of urea also showed

of this substance during a diet consisting exclusively of 300 g. sugar in a woman whose protein stock had been depleted because she had been on a low-protein diet for 81 days previously (figs 17 and 27).

(4) The slight nitrogen saving effect of NAPP *in a patient with uraemia* who had been on a very low protein diet for a long time may possibly also be based on the economical manner in which the body influenced the protein metabolism *prior to the use of the steroid*

(e) Two observations suggest that the quantity of protein in the diet is one of the factors determining the length of the interval between the administration of an anabolic substance and the nitrogen retention provoked

(1) Both after TPP and after NAPP the protein-saving effect was noticeable some 3 days earlier when the subject was on a diet containing 137 g. protein than when he was on an iso-caloric diet containing 22½ g protein per 24 hours (figs. 13 and 8).

(2) The maximal protein-saving with a diet containing 80 g. protein occurred as early as 2 *days after an injection of* TP (fig. 79) whereas with a diet containing 20 g protein *the same dose of TP caused the greatest* gain in nitrogen only after 4 days (fig. 75) In both cases, the greatest difference between the nitrogen and the sulphur balances was observed on the day of the maximal nitrogen retention It is possible therefore, that TP stimulates the synthesis of a low sulphur protein the more readily the more protein there is in the diet.

These observations may perhaps be explained on the basis of a difference in half life time of body proteins with varying quantities of protein in the diet. We have previously quoted the study by JEFFAY and WINZLER (1958) who in rats observed that the half life time of albumin was shorter the richer the diet was in protein

(f) After administration of MAD NAPP NAD and ANT no significant retention of water and sodium was observed, but 250 mg. TP caused a distinct decrease of the excretion of water and *sodium After administration of TPP no* retention of extracellular fluid could be demonstrated (table II) The rate of absorption may therefore be of importance for the influence on the excretion of water and NaCl. The slight reduction of the excretion of sodium and chloride which has been observed in the patient with uraemia after administration of 125 mg. NAPP (fig. 90) yet necessitates some caution in cases where retention of extracellular fluid represents a danger

(g) Although the data presented in table XXII (p 127) are global they nevertheless give an impression of the degree of saving *caused by injection of one dose of the* anabolic steroids (ANT has been administered *orally during one 24-hour period*).

4 *Excretion of creatinine*

In the course of some of the reported experiments differences have been observed of the quantities of creatinine excreted.

(a) A slow reduction during a protein-free diet, which *consisted of* 600 g. sugar per 24 hour (fig. 3) In 19 days, the creatinine excretion underwent a reduction of approximately 8 %.

tinctly whereas the excretion of nitrogen and sulphur remained unaltered (figs. 17 18, 19 and 20).

(4) After administration of an anabolic steroid the retention of phosphorus and potassium *was frequently greater than* might be expected from the figures on nitrogen. On a few occasions it could be observed that a slowly absorbed steroid (MAD and NAPP) immediately caused retention of phosphorus and potassium, followed by an increased excretion, while subsequently there developed a more protracted retention of phosphorus and potassium, which coincided with a reduced excretion of nitrogen. Relatively however even during this last-mentioned period the saving of phosphorus and potassium was greater than is in accordance with the composition of muscular protoplasm (figs. 68 69 72 and 73).

After administration of a steroid with more rapid action the excretion curve of phosphorus and potassium sometimes presented a split peak (figs. 80 and 81), probably caused by a partial coincidence of the two retaining influences.

(5) Measures which augment the production of creatine, such as administration of MAD will diminish the excretion of phosphorus, because part of the creatine is linked with energy-rich phosphate (QUERIDO KASSENAAR, SCHUURS and SELDENRATH, 1952). However this is definitely not the only cause of the relatively *high phosphorus retention after* administration of MAD for the excretion of phosphorus (and potassium), in comparison to nitrogen is also influenced more strongly by those anabolic steroids, which, due to the absence of a 17-methyl group in the molecule, do not stimulate the formation of creatine.

(6) There is a correlation between the excretions of calcium and phosphorus, which *appears from the following observations*

(a) The faecal excretion of phosphorus increases when the diet contains an excess of calcium (PETERS and VAN SLYKE, 1946). Accordingly a patient with uraemia, who was treated with calcium carbonate in order to prevent ketosis, showed a high faecal excretion of phosphorus (fig. 93).

(b) Measures, which enhance the retention of calcium in the course of a balance test, may have a similar influence on phosphorus owing to the deposition of calcium phosphate in the skeleton (PETERS and VAN SLYKE, 1946). The enhanced calcium excretion, which has been observed after administration of an excess of sodium chloride, may therefore affect the phosphorus excretion (fig. 88).

(7) Cells, which grow richer in glycogen incorporate potassium, which for this purpose moves from the extracellular fluid into the cells.

(8) It was once observed during a 24-hour period when the subject was in fever that phosphorus was retained, whereas nitrogen and sulphur showed an increased excretion (fig. 44).

When we review these eight points, we are convinced that the possibilities of influencing the excretions of phosphorus and potassium have not thereby been exhausted. This is readily understood when we realize that after protein, phosphate is

an increase during the first 24 hours after administration of TP It has been observed previously that no investigator has been able to demonstrate an increase of the urea clearance under the influence of anabolic steroids. It would appear likely therefore, that the increased excretion of creatinine is not to be attributed to a temporary improvement of the renal function. This view is also supported by a study by BÖNI and JUNG (1951) who after protracted administration of large *doses of TP observed very large excretions* of creatinine, which could not be the result of an improved clearance.

5 The proportions of nitrogen sulphur phosphorus and potassium as present in the excreta

In the above, we have repeatedly referred to the proportions of the weights of the protein catabolites excreted The excretion of sulphur phosphorus and potassium has many times been compared with that of nitrogen. Following the example of REIFENSTEIN ALBRIGHT and WELLS (1945) the excretions are represented in the figures by various scales The relationship of these scales is in accordance with the amount of the elements found in the muscular protoplasm.

It was found that on the whole there is a correlation between the alterations of the excretions of phosphorus and potassium under many different circumstances, *whereas the excretion of sulphur often* showed a completely different aspect. For this reason the excretion of sulphur will be considered separately

A. NITROGEN AND PHOSPHORUS OR POTASSIUM – Several facts observed in the course of the experiments described indicate that caution is necessary in drawing conclusions concerning the protein synthesis from the proportions in which nitrogen, phosphorus and potassium are present in the excreta.

(1) The rhythmic excretion of phosphorus in the course of a 24-hour period was quite different from that of nitrogen and sulphur (fig. 54)

(2) The excretion of phosphorus and potassium increased under the influence of change of diet in which protein and carbohydrate, which previously had been administered together were now given separately This finding is in accordance with *what has been observed in the case of* nitrogen and sulphur However when a similar alteration of the diet was carried out where protein and fat were concerned, the excretions of phosphorus and potassium were also increased, whereas the quantities of nitrogen and sulphur were not (figs. 58, 60 61 and 62).

(3) Addition of 20 g sodium chloride to a diet already containing 9 1 g. sodium chloride caused no perceptible alterations of the excretion of nitrogen and sulphur *whereas the excretions of potassium* and phosphorus were considerably increased even after equilibrium had been achieved between the intake and the excretion of sodium (figs. 86 87 and 88) In view of the fact that these findings had possibly been influenced by the NAPP administered previously another experiment was made in which 20 g. extra of sodium chloride per day was added to the diet for 3 days, after which the excretion of phosphorus and potassium increased dis

(3) In two experiments, addition of 20 g. sodium chloride per day to diets already containing a normal quantity of this salt, caused no alterations of the excretions of nitrogen and sulphur (figs. 17 18 and 85).

However in the experiments described, there were also circumstances characterized by alterations of the proportion of the nitrogen and sulphur excretions.

(1) In the first place, the N/S ratio of the waste matter was sometimes altered after a change of the quantity of protein in the diet.

(a) It appears from figs. 4 and 6 that the excretion of sulphur was relatively less than that of nitrogen during the first few days of a diet which consisted of 600 g. sugar exclusively This might be explained on the basis of a difference in clearance between the nitrogen-containing catabolites and the anorganic sulphates. For the sulphate there is a liminal value (SMITH, 1951), so that the reabsorption in the tubuli constitutes a larger part of the total glomerular filtration as the plasma concentration is less, whereas urea, on the other hand, diffuses to a less degree with lower concentrations in the tubuli. FAY and MENDEL (1925–1926) however starved dogs after first having given them a calorically complete, non-protein diet. Although the concentration of the protein metabolites undoubtedly increased after the change of the diet, the urinary excretion of sulphur was less than that of nitrogen.

(b) In the IInd experiment a diet containing 22½ g. protein was replaced by a nocaloric diet containing 137 g. protein. As the result of this change, the N/S ratio of the waste matter showed a considerable increase for a few days (table II). Due to the lack of an analysis of the food it was impossible to compare the balances of nitrogen and sulphur which would have been more accurate. This finding is in agreement with what FAY and MENDEL (1925–1926) observed in dogs which, after a period of fasting, received a normal diet in which the proportion between nitrogen and sulphur was 16.3 while the N/S ratio in the excreta was 41 on the 1st day and 36 during the second 24-hour period. This observation cannot be explained on the basis of a difference in clearance between nitrogen and sulphur either because that would lead us to expect precisely the opposite.

In view of the fact that, as stated above, the quantitative significance of sulphur as an electrolyte is probably slight, important alterations of the ratio of the N and S balances after a change of diet can presumably be explained only from alterations in the relative quantities of the body proteins.

WILSON (1925 1931) studied the influence of various changes of the diet on the excretion of nitrogen and sulphur On the basis of his observations, which are in agreement with the results of the above named experiments, he arrived at the conclusion that the body is able to accumulate proteins with different N/S ratios, the stability of the retained protein being greater the greater the relative content of sulphur PETERS and VAN SLYKE (1931) present objections to this theory which presupposes a labile circulating protein and a stable body protein, but they offer no other explanation.

Wilson s explanation would seem obvious if we presume, in agreement with Folin, that the tissue protein undergoes

the most important anion within the cell and that potassium is the preponderant intracellular cation (GAMBLE, 1947). Some of the above-mentioned influences on the excretions of phosphorus and potassium are therefore probably connected with the functions of these substances in the intra cellular fluid.

Most of the balance tests that have been described by REIFENSTEIN ALBRIGHT and WELLS (1945) concern observations over fairly long periods. As a rule the excreta were collected over six-day periods. The authors arrive at the conclusion that *after the administration of a number of injections of TP a retention of ni trogen sulphur phosphorus and potas sium develops which corresponds to the composition of muscular protoplasm* provided a correction of the phosphorus excretion is made in connection with the calcium balance. Deviations from this rule can be demonstrated only when the urine is examined in portions over shorter periods

The experiments reported show that practically always the retention of phosphorus and potassium after administra tion of an anabolic steroid was relatively greater than that of nitrogen. How ever the excretion of phosphorus and potassium depends on so many factors that from the proportion of the excretions of nitrogen and phosphorus or potassium no conclusions can be drawn concerning the manner in which anabolic substances promote the synthesis of protein.

B NITROGEN AND SULPHUR – The quantity of phosphorus in the intracellular fluid is 80 m.eq per litre but sulphur as far as we know plays only an insignificant part as an intracellular electrolyte. The extracellular fluid contains 1 7–2.6 m.eq phosphorus and 1 m eq sulphur per litre (GAMBLE, 1947).

There are some indications that the anorganic sulphates, present in the blood, must not be regarded simply as disintegration products of no value

(1) *After intraperitoneal administration* of labelled sodium sulphate to rats radioactivity can be demonstrated in various *tissues and proves to be linked to chon*droitine sulphuric acid (DZIEWIATKOWSKI, 1951)

(2) *Sulphates undergo partial reabsorp*tion in the renal tubuli (SMITH 1951).

It may probably be assumed, however that the quantitative significance of sulphur outside the protein molecule is slight, and it is therefore not surprising that some of the measures that have been taken in the course of the above-named experiments, and which gave rise to differences between the excretions of nitrogen and phosphorus (or potassium) caused no changes of the proportions between the quantities of nitrogen and sulphur found in the excreta.

(1) As a rule a certain uniformity was observed in the rhythmic renal excretion of nitrogen and sulphur When the food intake was divided regularly over the 24-hour period both substances followed the rhythm of the urine production (figs. 44 50 54).

(2) The excretion of nitrogen and sulphur did not increase as the result of a change of diet in such a way that fat and protein, previously administered together were now given separately (figs. 60 and 61).

scribed, can therefore be explained by the anabolism of the relatively low-sulphur proteins from the sex organs and the kidneys at the expense of sulphur rich serum albumin.

In other words, there is a shift of protein under the influence of anabolic steroids, which is not dependent upon the degree of nitrogen saving brought about. The earlier cited experiments of ABELS, NELSON, YOUNG and TAYLOR (1944) and of COOPER, RYNEARSON, MACCARTY and POWER (1951), who during administration of androgenic substances at first observed a decrease of the serum proteins in spite of a pronounced saving of protein, are in agreement with this observation.

The most marked differences between the excretions of nitrogen and sulphur occurred after administration of ANT in the IVth experiment the nitrogen balance did not change, whereas the sulphur balance during the first 24 hours after administration of ANT became more negative by 156 mg. This quantity of sulphur is liberated when approximately 16 g. serum albumin is transformed into body protein, the sulphur percentage of which is half that of serum albumin.

6. *Practical consequences for the dietetic treatment of patients with acute and chronic uraemia*

On the basis of the results of the balance tests described and of a number of data collected from the literature, the following directions can be given for the treatment of acute or chronic renal insufficiency

A. ACUTE ANURIA The purpose of the dietetic treatment is to restrict the protein catabolism. Owing to the short duration of the treatment maintenance of nitrogen equilibrium is not important.

(1) In order to limit ketosis, the diet must contain at least 100 g. carbohydrates per 24 hour

(2) The additional decrease of the protein catabolism, caused by administration of more than 100 g. carbohydrate, with protein-free diets, depends on a number of factors

(a) When an anuria occurs during a normal nutritional condition a quantitatively significant decrease of the protein catabolism, due to calorically richer diets, may be expected, which may be essential to the surviving of a patient with anuria which lasts several weeks (Vth experiment).

(b) When at the onset of an anuria the protein catabolism is slight, no significant decrease in the nitrogen excretion can be achieved with the aid of a calorically richer diet, although the decrease in percentages is considerable (IVth experiment)

(c) When the protein-sparing effect of extra carbohydrate is percentually as great with increased protein catabolism due to tissue damage, the effect of calorically richer diets in practically every case of anuria is quantitatively of great importance.

On the basis of these factors it is impossible to predict what degree of additional decrease of the protein catabolism can be achieved in every individual case of anuria with diets which contain more than 100 g. carbohydrate. The quantitative significance of this additional saving is often important, however because frequently an anuria develops in a person in a normal nutritional condition.

TABLE XXIII

APPROXIMATE AMINO-ACID COMPOSITION CALCULATED TO 16.0 g OF NITROGEN

protein	*cystine* (26.68 / S)	*methionine* (21.49 / S)
serum albumin	6.0	1.3
serum gamma globulin	2.6	1.1
muscle proteins	1.4	2.5
brain	1.7	2.4
heart	2.2	2.3
kidney	2.1	2.1
liver	1.7	2.3
lung	3.3	1.7
pancreas	3.2	1.9
spleen	2.7	1.7

no alterations apart from a normal process of wear. The investigations by Schoenheimer however have shown that the body proteins are continuously being built up and broken down with considerable differences in the transformation times of the proteins. In view of the dynamic state of the protein metabolism, relative decrease of the quantity of sulphur in the excreta can be explained by a shift of the body protein in which a high sulphur protein is formed out of protein containing less sulphur.

According to SHOHL (1939) muscle tissue contains relatively much sulphur. The relevant tables have been composed by the author from a number of older data. Later investigators have in a more accurate way determined the quantities of each of the amino-acids of which the various proteins are constructed (BLOCK and WEISS, 1956; MÜTING and WORTMANN 1954). Some of their findings are shown in table XXIII.

From this table it appears that serum albumin contains about twice as much sulphur as muscle protein and most of the other tissue proteins mentioned.

When as the result of a change of diet, the dynamic equilibrium of the protein metabolism is disturbed it will probably be the most active tissues which will adapt themselves first to the new situation. The relative paucity of sulphur in the excreta, after changing to a diet with an increased protein content, might therefore be explained by a relative increase of the serum albumin which is rich in sulphur and which, when the protein in the diet is decreased, is initially saved to a greater extent than most other body proteins, while serum albumin is also able to profit sooner from a high protein diet after a period of protein depletion.

(2) In the second place in a number of experiments a change of the proportion between nitrogen and sulphur in the waste matter has been observed after administration of anabolic steroids. The findings are listed in figs. 12, 17, 18, 31, 63, 67, 71, 75, 79 and 85. It was constantly found that, after administration of an anabolic steroid relatively more sulphur than nitrogen was excreted. Although as a rule the N/S ratios were lowest during the period of maximal nitrogen saving a distinct additional excretion of sulphur was also observed under conditions which caused no saving of protein (fig. 31).

From the review of the literature it can be concluded that the anabolic steroids do not cause a regularly distributed saving of protein, but that the sex organs and the kidneys as well benefit to a greater extent than the other tissues. The relatively increased excretion of sulphur after administration of the anabolic substances de-

losses of protein, which are suffered during calorically sufficient diets containing very little protein, may be compensated from time to time with blood transfusions, which in most cases are anyhow indicated to prevent a too severe uraemic anaemia.

(4) Very low-protein diets are always poor in calcium. By adding an excess of calcium salts to the diet, we obtain an additional excretion of phosphorus with the faeces.

(5) Mixtures of proteins of high biological value must be selected, the chemical composition of which differs little from the average composition of human protein. The catabolic influence of an excess of one or more amino-acids is thereby limited. It is not possible to give more accurate directions for the selection of proteins in the diet there are too many lacunae in our knowledge concerning the quantities of the amino-acids required by the human organism, and concerning the biological value of the various proteins.

(6) Protracted bed rest is to be avoided when possible.

(7) By adopting optimal dietetic prescriptions the catabolism of protein can be limited to such a degree, that little or no saving of nitrogen can be expected from androgenic steroids. When the observed sulphur loss is actually to be ascribed to synthesis of protein in the sex organs and kidneys at the expense of serum albumin, administration of androgenic substances might even be contraindicated.

From the experiments described it does *not emerge clearly how large the* addition must be The caloric value, above which no significant decrease of the protein catabolism can be obtained grows smaller as the diet contains less protein. For a diet that contains some 20 g. protein this caloric value was a little higher than for a diet on which the body weight remained stable With a protein free diet, the protein saving will be probably practically optimal when the body weight remains stable.

(3) According to the literature there is no *objection against making fat part of the* diet. There are some indications that fat is indispensable for an optimal saving of protein but this is only true of calorically incomplete diets. The influence was most pronounced when the diet contained little protein

(4) *The food must be administered in* many portions This is also true for parenteral feeding.

(5) The faecal excretion of phosphorus can be increased by adding calcium to the diet.

(6) Protracted bed rest is to be avoided when possible.

(7) Androgenic steroids have a greater protein-saving effect the less the body protein stores have been depleted. For this reason a favourable influence on protein synthesis can be expected in acute anuria. It is possible however that this is associated with a shift of protein in the body which may result in an increase of the anorganic *sulphur in the blood.*

B CHRONIC URAEMIA – (1) In order that *ketosis* be restricted the diet must contain at least 100 g. carbohydrate.

(2) As in Holland patients with chronic uraemia are forced to use a low *protein diet, the protein catabolism is* practically always slight. For this reason an increase of the caloric value of the diet above that of 100 g. carbohydrate will quantitatively have a slight protein-sparing effect. In the long run this may yet be important, however because the diet in question must be kept indefinitely The *aim must therefore be a caloric value of* the diet with which the body-weight can be kept stable Carbohydrates and isocaloric quantities of fat presumably have the same protein-saving significance. There are a few indications that fat is indispensable for optimal nitrogen saving this has been demonstrated, however for *calorically insufficient diets only* and the effect was more pronounced the less protein the diet contained

(3) Because with some 20 g. protein in the diet under otherwise optimal conditions it is hardly possible to maintain nitrogen equilibrium the diet must, if possible, contain more protein Therefore no attempt should be made to keep the urea level of the blood normal.

However part *of the pathological* phenomena associated with uraemia may often be combatted with success by prescribing calorically sufficient diets that contain very little protein It would appear that the disadvantages of a continuously negative nitrogen balance are often less important than the advantages to be *obtained* with such a diet. The small daily

protein there is in the diet. In diets very poor in protein this caloric value is probably equal to the number of calories necessary for maintaining body weight.

(4) The saving of proteins by carbohydrates is greater if they are administered together with the protein. Animal experiments showed that for fat this time-linked factor is not present.

Some data from the literature may warrant the impression that NaCl produces a significant saving of protein. In the studies concerned, however salt free diets were compared with diets containing excessive amounts of NaCl (section V).

During strict rest in bed maintenance of nitrogen equilibrium is only possible if the diet is rich in proteins (section VI).

The seventh section discusses the application of data from nutritional physiology to the treatment of patients with renal failure. Opinions differ considerably on the caloric value of the diet and the amount of protein the diet has to contain in cases of uraemia. The arguments of those workers who doubt the considerable saving of protein brought about by diets of adequate caloric value appear to be based on experiments that are unsuitable for studying the value of the diet in cases of uraemia and have been interpreted erroneously by others.

In cases with severely impaired renal function one is frequently forced to reduce the amount of proteins to a value insufficient for maintaining nitrogen equilibrium. The disadvantages of the slight daily loss of protein appear to be less important than the advantages that may be gained in this manner by reducing the level of protein catabolites in the blood.

Section VIII of the literature data deals with the anabolic influence of androgenic steroids. These data lead to the following conclusions

(1) On protracted administration of androgenic steroids the protein-saving effect decreases.

(2) The protein synthesis brought about by androgenic substances usually increases with increasing protein levels of the diet and higher caloric value of the same. The protein-anabolic effect is reduced in rats if their weight has considerably decreased.

(3) Not all tissues benefit equally from the administration of androgenic steroids. The greatest synthesis is found in the genital organs, followed by the kidneys. There is evidence that the protein synthesis in the genital organs and kidneys takes place at the expense of protein in other parts of the body if necessary

(4) Some nor-testosterone derivatives are to be preferred under some circumstances since they combine a distinct anabolic effect and a moderate androgenic effect, while the retention of sodium is small.

The second part of the thesis (C) gives a description of personal experiments. The 16 balance tests, 2 of which were made in a patient with uraemia, are first briefly described This is followed by a review of the various methods of determination. Usually nitrogen, sulphur phosphorus, potassium and sodium were determined both in the food and excreta. Sometimes the composition of the food was estimated with the aid of a table of foods.

Secondly the experiments are described

D

SUMMARY

This thesis deals with the quantitative importance of several factors for the metabolism of proteins

In the first part (B) data from the literature have been described The first section concerns the methods for the determination of the nitrogen balance, in which detailed notes are made on marking of faeces, since it has not been possible to find a reliable method for marking that does not interfere with the colorimetric determination of phosphorus and the nephelometric determination of sulphur

The third section describes some aspects of protein metabolism related to the subject. The following conclusions have been reached

(1) The degree of protein saving that can be expected after certain dietetic or other measures depends on the state of nutrition Part of the proteins in the organism can be easily broken down Once this protein deposit has disappeared protein catabolism decreases sharply and the quantitative effect of protein saving influences becomes small

(2) Under certain circumstances considerable alterations in the quantitative proportions of protein catabolites in the urine develop rapidly Schoenheimer's experiments explain these observations. There is a dynamic equilibrium between the proteins of the organism and the nitrogen-containing substances in the food This brings about continual changes in the cytoplasm while there is also regular exchange between the tissues. Proteins from the liver and serum albumin participate more actively in the dynamism of the protein metabolism than do other proteins in the body The liver is capable of rapidly releasing protein which possibly is partly at the expense of formation of sulphur rich serum albumin Under more favourable conditions the hepatic proteins and serum albumin are the first to benefit from the greater amount of basic material available.

In section IV quantitative data on the protein-saving effect of carbohydrates and fats are given These lead to the following conclusions

(1) For optimal saving of protein an amount of 100 g. carbohydrates per day is indispensable.

(2) In general it has appeared that above 100 g. of carbohydrate as great a saving of protein can be achieved with carbohydrates as with iso-caloric amounts of fat.

(3) There is a caloric value above which no further significant saving of protein may be expected. This is lower the less

caloric value of the diet depends on the initial protein catabolism. This is clearly demonstrated by a comparison of experiments IV and V Experiment IV was carried out in a woman who after being on a diet poor in proteins for 81 days, had lost approximately 400 g. of protein due to a continuously negative nitrogen balance. Consequently the protein catabolism had decreased to a very low value the amount of variable nitrogen excreted was 3.3 g. per 24 hours. This fell to 2.4 g. when the low-protein diet was changed to a diet consisting of 100 g. sugar exclusively By increasing the amount of sugar to 300 g. daily the excretion of variable nitrogen with the urine was further reduced to 1 7 g., corresponding to 11 6 g. protein

The Vth experiment was carried out in two young men (A and B). The pattern of the diet test was as follows

Firstly both subjects on a 5-day normal diet containing 80 g. protein per day (the daily excretion of variable nitrogen was then 12 g. approximately)

Secondly exclusively sugar A receiving 100 g., B 400 g. daily

(An interval of nonstandardized normal eating.)

Thirdly both subjects again on a 5-day normal regimen containing 80 g. protein per day

Finally exclusively suga A receiving 400 g and B 100 g. daily

The extra saving of protein on a diet of 400 g. sugar was far greater than that obtained in experiment IV by increasing the amount of sugar from 100 to 300 g. daily as appears from the figures n table XIX. Therefore, the absolute value of the extra sa ing of protein by increasing the amount of sugar bove 100 g. per 24 hours was far greater for the men who had previously received a normal diet than for the woman who had been on a low-protein diet for a long time In percentages, however there was a similarity between the amount of extra saving of protein obtained in both tests.

Under optimal conditions nitrogen equilibrium could hardly be obtained with diets containing approximately 20 g. protein of high biological value (table XXI).

In the first experiment a diet consisting exclusively of sugar was divided alternately over six and over two meals per day The excretion of protein catabolites was lower during the period of frequent meals. Quantitatively this gain was of little significance.

(3) The diet in experiments VI to IX inclusively consisted of nutrients containing only protein carbohydrate or fat.

The following observations were made

(a) An excess of methionine led to poorer use of other amino-acids from the diet (exp. VI).

(b) Isocaloric replacement of a part of the fat by carbohydrate caused a distinct saving of nitrogen during the administration of a diet that initially was solely composed of fat and protein (exp VIII).

(c) With mixed diets extra nitrogen was lost after administration of carbohydrate and protein separately This time factor did not exist for fat and protein (exps. VII and IX).

(4) The protein-sa ing effect of *androgenic hormones* was studied n experiments II III, IV and X to XIV inclusively In table XXII a number of data on the saving of protein obtained by androgenic substances have been collected. They show the following facts

separately The findings are always presented in tabular form and have also been recorded in a general graph Always, a number of data have been presented on a larger scale in separate graphs.

Finally a number of conclusions have been drawn usually from combined observations These conclusions are as follows

(1) The faecal excretion of nitrogen was approximately 0 8 g. per 24 hours on a varied diet. Usually it has been assumed that this value amounts to 1 3 g. daily The faeces of a patient with uraemia who received a varied diet containing only 18 or 15 g. of protein contained 1.3 g. nitrogen daily on the average Faecal nitrogen excretion was therefore approximately half *the ingested amount.*

The data of all nitrogen determinations in the faeces are presented in table XVIII.

(2) In the first 5 experiments a study was made of the decrease in *protein catabolism* during administration of a diet containing a small amount of or no protein Though from the IInd to IVth experiment inclusively anabolic steroids were administered several times the conditions of the experiments were chosen in such a manner that the effect of the diet exclusively could be *determined.*

The first 3 experiments are comparable since they involved the same subject. In the first experiment the diet consisted of 600 g. *sugar only in the second the diet was a* varied one supplying 3190 calories and containing 22½ g protein and in the IIIrd experiment the diet supplied 500 calories and contained 20 4 g. protein. A number of data have been compiled in table XX. From these it appears that on the 19th day of the experiment 3.5 4 4 and 6 7 g. nitrogen respectively were excreted. The variable nitrogen (urea and ammonia) at this day *amounted to 2.2, 2.9 and 5.5 g.,* respectively Therefore the protein catabolism showed practically the same decrease during administration of the diet of adequate caloric value containing 22½ g. protein and during administration of the protein-free diet supplying 2400 cal. The significance of this fact becomes clear when calculating the rise in blood urea that would have taken place during the first 18 days of the experiment, provided no urine had been produced. In this case the blood urea level would have increased by 2130 2350 and 4490 mg. per liter respectively By increasing the caloric value from 500 to 3190 per 24 hours the protein *catabolism was therefore practically halved* in the case of diets containing approximately 20 g. protein.

The significance of the *caloric value* of the diet for the degree of protein catabolism was further studied in the IIIrd experiment. The diet contained approximately 20 g. protein, the caloric value was 500 per 24 hours for 66 days and then was increased by steps of 500 cal. until a value of 3500 cal. daily was reached. The increase in caloric value was proportionally less *significant* the higher the caloric value of the diet. The curve *of the nitrogen balance* had an asymptotic shape, as appears from fig 100 The caloric value above which no *distinct improvement* of the nitrogen balance could be observed was somewhat higher than the number of calories necessary for maintaining weight. Nitrogen equilibrium was obtained at 3000 cal

From the above facts it appears that the protein catabolism becomes less the more *the caloric value of the diet increases.* The quantitative value of an increase in

slightly negative, even on these diets with their extremely low content of protein. Therefore the depletion can usually be compensated by blood transfusions. On account of refractory anaemia these are frequently necessary anyhow

(b) The protein catabolism, calculated from the excretion of urea and that of ammonia, appeared to be very slight. The excretion of variable nitrogen was 1 4 g. per 24 hours, corresponding to 8 4 g. protein.

(c) Presumably on account of this low protein catabolism the protein-saving ef fect of Durabolin was hardly noticeable.

(d) The faecal excretion of nitrogen and sulphur constituted a significant part of the total excretion The faecal excretion of phosphorus was more than 3 times higher than the renal excretion of this element, due to the orally administered calcium carbonate There was no increased faecal excretion of potassium.

Finally the practical consequences for dietetic treatment of patients with renal disorders have been discussed, based on data from the literature cited and the results of personal experiments. Measures for short term treatment of acute an uria have to be entirely directed at limiting the protein catabolism, whereas in long-term treatment of chronic uraemia attention also has to be given to maintenance of the nitrogen balance. Rest in bed for long periods has to be avoided.

(a) Androgens had a more rapid and stronger protein-saving effect with diets containing 80 g. protein than with diets containing only 20 g. protein daily (exps X XI XII and XIII)

(b) The nitrogen saving effect of androgenic steroids was lower the longer a low-protein diet of insufficient caloric value had been used. During a state of protein depletion in which protein catabolism was very low no saving of nitrogen was observable after administration of a highly active anabolic steroid (exp IV)

(c) 19-nor androstenolone phenylpropionate (Durabolin Organon) and testosterone phenylpropionate ('TPP Organon) produced a practically equal saving of protein which was considerably more than could be obtained with methyl androstene diole ('Neosteron Organon) Ethyl nor testosterone (Nilevar Searle) was administered during a state of protein depletion Therefore the degree of protein saving could not be compared with the influence of the above mentioned testosterone derivatives. No saving of protein was observed but a considerable shift in the proteins of the organism is suspected since the excretion of sulphur showed distinct increase

(5) A number of observations on changes in the excretion of *creatinine* have been mentioned separately (pp 128 129 130)

(6) The excretion of protein catabolites did not change after addition of 20 g. *sodium chloride* to diets that contained a normal quantity of NaCl (exps. III and XIV).

(7) During *rest in bed* a negative nitrogen balance was invariably observed. Nitrogen equilibrium was not obtained even with diets of adequate caloric value that contained 80 g. protein of high biological value daily (exps. X and XII)

(8) The quantitative ratio of protein catabolites in the excretions is dealt with in two sections

Several examples illustrate that changes in the excretion of *phosphorus and potassium* frequently do not permit conclusions on protein metabolism since these elements also participate in other important functions of the organism. For a study of the protein metabolism the proportion of nitrogen and *sulphur* in the excretions is more important. Great changes in this proportion that cannot be explained by the administered quantities of these elements, almost certainly testify that the quantitative proportions of the proteins in the organism have changed A reduction as well as an increase of the quantity of proteins in the diet always temporarily provoked a relatively large retention of sulphur After administration of androgenic steroids an extra loss of sulphur was always noted, even if no nitrogen saving effect could be observed. This can be explained from the synthesis of proteins in the genital organs and kidneys at the expense of serum albumin, which contains a greater amount of sulphur

(9) Experiments XV and XVI were made in a patient with uraemia who received a diet of adequate caloric value containing 18 or 15 g proteins respectively plus 3 g. calcium carbonate daily The protein saving effect of Durabolin was also studied. The results lead to the following conclusions

(a) Nitrogen equilibrium could not be obtained. The total nitrogen excretion amounted to approximately 3 5 g. daily corresponding to 21 g. protein Consequently the nitrogen balance was only

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F

TABLES I–XVII

TABLE I

URINE					FAECES			BLOOD			
S mg/hr	P mg/hr	K m eq/hr	N m eq/hr	Cl m eq/hr	N mg/hr	S mg/hr	P mg/hr	urea mg/L.	K m.eq/L.	Na m eq/L.	Cl m eq/L.
[illegible] 7	37 5	2.5	4 5	3.7	11	0 9	2.5				
17 4	33 8	2 1	1 4	1 8	11	0 9	.5				
16 3	21.1	1 4	0.8	1.3	11	0 9	2.5		3 6	137	100
13 5	19 3	1.0	0 4	1.2	11	0.9	2 5				
13 3	14 6	0.7	0 2	1.0}	11	0.9	2 5				
13 3	14 6	0 7	0.2	1.0}	11	0 9	2 5				
13 9	14 1	0 8	0 1	0 8	11	0 9	2.5				
14 2	11 4	0.7	0.1	0.9	11	0 9	2.5				
14 1	13 1	0.7	0 1	0 8	11	0 9	2 5				
13 8	9 8	0 5	0.1	0.7	11	0 9	2.5	131	4 3	136	99
13 8	10 6	0 5	0.1	0 6	9	0 8	1.8				
12 5	10 3	0 4	0.2	0.5	9	0 8	1.8				
12 4	10 4	0.5	0 1	0 5	9	0 8	1.8				
13 8	10 8	0 5	0 1	0 5	9	0.8	1.8				
12.3	8 8	0 5	0 1	0 3	9	0 8	1 8				
12.5	10 3	0 4	0.1	0 3	9	0 8	1.8				
11 8	6 1	0 3	0 1	0 2	9	0.8	1.8				
10 4	6 8	0 3	0.1	0 3	9	0 8	1.8				
10 6	6 0	0 4	0.1	0 5	9	0 8	1.8				
14 3	5.8										
14 8	9 6										
14 5	7 7				9	0.8	1.8				
12 8	11 2										
13 3	9 8										
13 9	6 3										
13 2	3 0										
11 6	2 8										
12 9	6 4										
13 8	8.1										
11 5	8 1										
12 8	7 0				9	0.8	1.8				
7 1	5 2}										
7 1	3 2}										
13 0	7 4										
13 7	0 3										
13 8	3 1										
11 8	9 5										
10 8	7 3										
10 5	7 2										
11 1	5 7				9	0.8	1.8				
9 4	4										
11 1	5 9										
10 8	3 5										
12	0 8										
11 1	1										
10 3	7										
10 5	4 3										
9 8	3 3										
10 7	3 8										
9	3 5										
10 3	3 9										
10 4	2 6										
10 0	3 3										
					8	0 8	1.8				

TABLE I

Experiment I

date 1953	hour	FOOD calories /day	K m.eq /h	N m.eq /hr	Cl m.eq./hr	DETAILS	URINE body-weight kg	quant ml /day or 3 hrs.	tot. N mg /hr	urea mg N./hr	amm. mg N/hr	creat. mg N/hr
Sept						6 × 100 g.						
20		400	0.005	0.1	0.13	sugar		1740	530	460	23	27
21							86.7	1815	330	280	4	27
22						"	86.1	1615	260	210	20	25
23						"	85.2	1505	220	170	18	26
{24							85.4	1285	190	140	19	27
{25							85.2	1285	190	140	19	27
26							85.1	1400	210	140	19	25
27							85.1	1400	190	130	19	26
28							84.7	1095	180	120	19	27
29							84.4	1180	180	130	18	28
30							84.[illegible]	1270	170	110	19	26
Oct.												
1							84.3	1540	170	120	17	25
2							84.1	1605	150	100	16	25
3						2 × 300 g. sugar	83.8	1345	160	110	17	25
4							83.5	1280	160	110	15	25
5							83.5	1620	160	110	15	4
6						6 100 g. sugar	83.4	1145	130	80	13	25
7							82.6	1425	140	90	13	24
8						8 × 75 g. sugar	83.0	1585	130	90	13	25
9	0–18						82.8					
	18–21		0.007	0.13	0.17	bed rest		317	180	140	13	28
	21–24							305	180	140	15	27
	18–24							622	180	140	14	28
10	0–3							[illegible]77	160	120	15	25
	3–6							47	160	120	16	25
	6–9							277	170	120	18	26
	9–12							348	180	130	16	27
	12 15							258	150	110	13	23
	15–18							307	170	120	15	25
	18–21							361	170	130	15	26
	21–24							260	160	120	13	24
	0–24							2335	160	120	15	25
11	{0–3							202	100	70	5	17
	{3–6							202	100	70	5	17
	6–9							260	150	110	17	24
	9–12							362	180	140	17	27
	1 15							309	170	130	15	24
	15–18							310	170	130	15	26
	18– 1							229	150	110	13	24
	21 24							231	150	110	14	24
	0–24					"		1903	150	110	13	23
12	0–3							184	130	90	15	23
	3–6							225	140	100	17	24
	6–9							184	130	90	17	23
	9–12							305	160	120	17	27
	12 15							287	140	100	13	25
	15–18							315	140	100	15	25
	18–21							21	130	100	9	4
	21–24							191	120	80	13	24
	0–24							1899	140	100	25	25
13	0–3							155	120	80	4	23
	3–6							160	120	80	16	23
	6–9							208	120	80	15	22
	0–9							523	120	80	23	23

TABLE II

FAECES					BLOOD						
N mg/hr	S mg/hr	P mg/hr	K m eq/hr	Na m eq/hr	date blood examination	urea mg/L	total protein serum g/L	albumen g/l	pseudo-globulin g/l	eu-globulin g/L	Hb. g %
35	3 24	6 5	0 46	0 07							
35	3 24	6.5	0 46	0 07	April 27	329	76 7	49.1	8 0	19 6	16.4
35	3 24	6.5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07	May 7	229					16.3
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0.07							
35	3 4	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0.46	0 07							
35	3 24	6 5	0 46	0 07	May 24	209					15 8
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 4	6 5	0.46	0 07	June 9	197	73 8	42.4	11.8	19 6	16 4
35	3 24	5 6	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0.07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07	June 23	1[illegible]					16 9
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07							
35	3 24	6 5	0 46	0 07	July 9	180					16 0
33	3 24	5	0 46	0 07							
33	3 24	6 5	0 46	0 07							
33	3 24	6	0 46	0 07							
35	3 24	6 5	0 46	0 07	July 22	174	73 1	43 6	12 5	17.0	15.8
39	80	16 9	0 3	0 07							
39	4 80	16 9	0 53	0 07	July 26	266					15.7
39	4 80	1 9	0 3	0 07							
39	80	9	0 55	0 07	Aug 2	305					
39	4 80	1 9	0 5	0 07							16.1
39	80	16	0 55	0 07	Aug 8	565					
											16 0

TABLE II

EXPERIMENT II

date 1956	FOOD calories /day	N mg /hr	MED	body-weight kg	URINE quant ml /h	tot N mg./hr	urea mg.N/hr	creatinine mg.N/hr	S mg./hr	P mg./hr	K m-eq./hr	Na m-eq./hr
April												
4–26	3190	150		85.7	60	390	322	27.8	22.1	27.0	2.16	3.70
27–29				84.5	47	210	160	27.2	17.5	24.4	1.92	3.46
May												
30–2				85.5	62	161	112	27.2	15.6	22.2	2.08	3.44
3–5				85.4	48	140	85	26.3	14.8	21.2	2.06	3.65
6–8				84.9	52	158	105	26.1	14.2	21.0	2.10	3.45
9–11				85.15	61	150	99	25.4	14.9	21.4	2.07	3.85
1 14				85.25	69	148	95	25.4	14.1	24.0	2.20	3.30
15–17				84.9	54	147	93	24.7	14.7	19.1	2.00	3.15
18–20				84.6	59	147	93	24.8	15.9	18.8	1.88	3.55
21 23				85.0	52	154	101	24.9	15.2	19.6	.13	3.30
			May 24 NAPP									
24–26			125 mg. 1 ×	84.9	56	143	90	4 7	15.9	19.6	2.05	3.40
27 29				84.9	49	118	67	24.3	15.5	18.4	1.67	.85
June												
30–1				85.4	62	121	80	23.6	15.0	17.5	1.80	3.75
2–4				85.5	58	133	89	23.6	14.9	17.4	39	3.71
5–7				85.15	69	153	101	24.2	14.4	21.0	.25	3.82
			June 8 MAD									
8–10			125 mg. 1 ×	84.5	59	140	87	.3.3	14.8	20.2	1.76	2.81
11 13				84.95	54	131	80	23.5	13.6	20.4	1.67	2.40
14–16				85.2	73	134	88	24	12.1	21.0	.05	4.06
17 19				84.6	53	132	87	23.4	11.7	21.0	1.82	3.95
20–22				85.1	66	129	88	23.2	11.6	21.0	1.92	3.76
			June 23 TPP									
.3–25			125 mg. 1 ×	85.15	57	123	81	23.4	1 7	17.9	1.54	4.29
26–28				85.8	58	11	72	23.3	13.8	18.0	1.61	3.85
July												
29–1				85.2	51	115	72	22.9	11.4	14.0	1.63	3.35
2–4				84.9	49	124	79	22.9	10.7	22.4	1.70	3.00
5–7				85.4	66	126	79	23.0	11.6	21.5	2.15	4.44
			July 8 NAPP									
8–10			125 mg. 1	85.3	53	121	74	23.0	11.4	17.3	1.77	3.75
11 13				85.5	55	110	67	1 6	11.0	18.0	1.28	3.12
14–16				85.85	53	102	58	22.4	11.2	18.5	1.71	3.55
17–19				85.9	54	119	83	.3.6	11.5	1.8	2.28	4 4
20–22				85.3	55	132	75	23.6	11.2	21.1	2.00	3.55
23–25	3190	91		85.3	53	870	736	28.5	28.9	45.6	2.64	7.65
.6–28				86.3	47	730	650	29.4	46.8	55.0	2.46	8.40
29–31				85.7	42	785	720	30.3	49.5	57.5	1.92	6.18
August												
1 3				86.5	55	838	780	30.2	53.0	60.0	1.78	7.57
4–6				87.2	64	825	765	30.6	60.5	61.5	1.78	7.70
7 9				87.15	52	840	782	31.1	49.5	61.0	2.30	7.70

TABLE II

FAECES					BLOOD						
N mg/hr	S mg/hr	P mg/hr	K m.eq/hr	Na m.eq/hr	date blood examination	urea mg/l	total protein serum g/L	albumin g/L	pseudo-globulin g/L	eu-globulin g/L	Hb. g %
38	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07	Aug. 14	644	72.8	47.9	11.3	13.6	15.8
38	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07	Aug. 27	615	77.7	47.4	13.1	17.2	15.6
39	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07	Aug. 31	660					
39	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07							
39	4.80	16.9	0.55	0.07	Sept. 12	580	74.0	46.7	10.8	16.5	15.8

TABLE II

EXPERIMENT II

date 1956	FOOD calo-ries /day	FOOD N mg./hr	MED	body-weight kg	URINE quant ml./hr	URINE tot N mg./hr	URINE urea mg. N/hr	URINE creatinine mg. N/hr	URINE S mg./hr	URINE P mg./hr	URINE K m eq /hr	URINE Na m eq
10–12				87..	53	875	815	32.6	50.0	62.0	2.25	9.70
			Aug. 13 TPP									
13–15			125 mg. 1 ×	87.1	55	835	778	32.3	47.5	60.0	1.96	8.11
16–18				87.45	48	785	722	31.7	45.5	52.0	1.68	6.55
19–21				88.0	63	840	765	32.0	49.0	61.5	2.60	9.40
22–24				86.8	63	860	795	31.2	54.0	65.0	2.65	7.72
25–27				86.8	55	840	760	32.1	49.5	55.0	2.38	6.30
28–30				87.0	52	861	790	33.1	56.0	51.5	2.06	8.30
			Aug. 31 NAPP									
Sept. 31–2			125 mg. 1 ×	87.0	53	770	695	32.7	53.0	60.0	1.74	7.90
3–5				87..5	57	791	709	33.5	49.0	53.0	1.90	9.60
6–8				86.8	49	855	770	33.7	55.0	59.5	2.50	7.80
9–11				86.8	54	905	825	33.5	55.0	63.0	2.64	7.70

TABLE II

FAECES					BLOOD						
[illegible] mg/hr	S mg/hr	P mg/hr	K m eq./hr	N m.eq./hr	date blood exami-nation	urea mg/L	total protein serum g/L	albumin g/l	pseudo-globulin g/L	eu-globulin g/L	Hb g %
[illegible]	4 80	16 9	0.55	0 07							
[illegible]	4 80	16 9	0 55	0 07	Aug 14	644	72.8	47 9	11.3	13 6	15 8
[illegible]	4 80	16 9	0 55	0 07							
[illegible]	4 80	16 9	0 55	0 07							
[illegible]	4 80	16.9	0 55	0 07							
[illegible]	4 80	16 9	0.55	0 07	Aug 27	615	77 7	47 4	13 1	17.[illegible]	15 6
[illegible]	4 80	16 9	0 55	0 07							
[illegible]	4 80	16 9	0 55	0 07	Aug 31	660					
[illegible]	4 80	16 9	0 55	0 07							
[illegible]	4 80	16 9	0 55	0 07							
[illegible]	4 80	16 9	0 55	0 07	Sept. 12	580	74 0	46.7	10.8	16 5	15 8

TABLE III

EXPERIMENT III

date 1938	calories /day	calorie /kg body-w	N mg./hr	S mg./hr	P mg./hr	K m.eq./hr	C m.eq./hr	DETAILS	body weight kg
	FOOD								
Sept.–Oct.									
29–1	500	5 4	136	9.6	18.0	1.2	0.93		93 35
2–4									90 55
5–7									89.6
8–10									88.3
11 13									87 45
								Oct. 14	
14–16								NAPP 50 mg 1x	86 65
17 19									86 3
20–22									85 45
23–25									84 6
26–28									83 6
29–31									82.55
N									
1 3									82.2
4–6									81.5
7–9									80 6
10–12									80.2
								Nov 13	
13								NAPP 50 mg. 1	79 25
14									79 30
15									79 2
13–15									
16									79.05
17									78.7
18									78 5
16–18									
19–21									78.4
22 24									77.5
25–27									76.9
28–30									76 0
Dec.									
1 3									75 55
	Dec. 4								
4–6	1000	13.3	139	9 9	18 6	1.2	0 96		75.2
7 9									75 0
10–12									74 25
	Dec 13								
13–15	1500	20.4	141	10	19 1	1.2	0.98		73 6
16–18									73.6
19–21									72.9
	Dec 22								
22–24	2000	27 5	144	10.5	19 6	1.2	1 01		72.75
25–27									73 2
28–30									72 8
Jan. 59	Dec. 31								
31 2	2500	34 3	146	10 8	20.1	1.2	1.03		73 0
3–5									73 3
6–8									73 4
	Jan. 9								
9–11	3000	40.8	149	11 1	20.7	1.2	1 05		73 45
12 14									73 6
15–17									74 0

TABLE III

[illegible]

URINE									FAECES				
[illegible]	tot. N mg/hr	urea mg N /hr	ammon. mg N /hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m eq. /hr	C m eq /hr	N mg/hr	S mg/hr	P mg/hr	K m eq /hr	C m eq. /hr
[illegible]	535	485	18.6	28.9	35.6	40.5	2.63	0.37	12	1.4	10.1	0.26	0.70
73	459	410	19.7	27.0	29.0	32.1	1.79	0.33	12	1.4	10.1	0.26	0.70
[illegible]	364	321	17.8	26.5	25.2	26.2	1.62	0.34	12	1.4	10.1	0.26	0.70
[illegible]	311	270	15.2	27.2	22.0	26.0	1.56	0.38	12	1.4	10.1	0.26	0.70
57	306	271	15.1	26.6	21.0	22.6	1.68	0.37	12	1.4	10.1	0.26	0.70
[illegible]	280	230	16.2	26.5	19.6	20.6	1.28	0.345	12	1.4	10.1	0.26	0.70
[illegible]	269	234	16.0	25.0	20.0	19.9	1.44	0.365	12	1.4	10.1	0.26	0.70
[illegible]	256	226	12.8	25.4	19.6	16.4	1.67	0.36	12	1.4	10.1	0.26	0.70
[illegible]	252	214	10.7	23.9	19.6	17.2	1.80	0.34	12	1.4	10.1	0.26	0.70
[illegible]	251	212	11.7	23.9	18.4	16.8	1.62	0.37	12	1.4	10.1	0.26	0.70
62	230	188	13.1	23.1	17.8	15.1	1.24	0.375	12	1.4	10.1	0.26	0.70
[illegible]	240	208	13.1	23.3	18.0	18.8	1.62	0.36	12	1.4	10.1	0.26	0.70
71	250	214	12.6	23.2	18.9	17.1	1.72	0.38	12	1.4	10.1	0.26	0.70
[illegible]	236	206	13.6	23.2	19.2	16.6	1.48	0.38	12	1.4	10.1	0.26	0.70
[illegible]	236	206	14.4	22.6	18.5	17.1	1.36	0.455	12	1.4	10.1	0.26	0.70
[illegible]	228	192	15.0	21.5	17.4	13.6	1.38	0.44	12	1.4	10.1	0.26	0.70
[illegible]	232	207	16.6	22.2	18.6	19.9	1.12	0.41	12	1.4	10.1	0.26	0.70
[illegible]	235	208	17.8	23.1	18.8	19.6	1.24	0.44	12	1.4	10.1	0.26	0.70
	229	201	16.5	22.3	18.3	17.7	1.25	0.43	12	1.4	10.1	0.26	0.70
[illegible]	223	189	14.2	1.6	17.8	16.4	1.32	0.355	12	1.4	10.1	0.26	0.70
[illegible]	223	196	12.8	22.3	21.4	12.9	1.32	0.38	12	1.4	10.1	0.26	0.70
[illegible]	215	190	12.5	22.1	19.2	13.9	1.19	0.37	12	1.4	10.1	0.26	0.70
	222	195	13.2	22.0	19.5	14.4	1.28	0.37	12	1.4	10.1	0.26	0.70
71	233	201	12.5	22.1	19.5	14.4	1.80	0.385	12	1.4	10.1	0.26	0.70
[illegible]	226	200	12.2	20.9	19.8	16.6	1.60	0.44	12	1.4	10.1	0.26	0.70
[illegible]	221	198	11.6	20.9	16.8	15.8	1.47	0.44	12	1.4	10.1	0.26	0.70
[illegible]	226	199	13.2	20.9	16.2	14.3	1.32	0.46	12	1.4	10.1	0.26	0.70
63	236	204	13.9	19.8	15.4	14.0	1.27	0.395	12	1.4	10.1	0.26	0.70
[illegible]	194	166	11.0	20.9	14.8	13.8	1.45	0.38	16	1.6	9.7	0.38	0.70
[illegible]	186	162	10.8	21.0	15.0	12.9	1.38	0.425	16	1.6	9.7	0.38	0.70
63	200	177	12.7	19.5	15.4	14.2	1.15	0.455	16	1.6	9.7	0.38	0.70
[illegible]	179	154	12.9	1.0	17.0	10.7	1.34	0.43	18	1.8	9.7	0.25	0.87
73	206	179	13.4	20.6	15.2	15.3	1.54	0.42	18	1.8	9.7	0.25	0.87
70	187	145	12.4	20.1	17.7	15.8	1.41	0.44	18	1.8	9.7	0.25	0.87
[illegible]	174	147	14.8	20.2	14.6	14.3	1.20	0.30	18	1.8	9.7	0.25	0.87
73	162	177	13.8	20.0	13.1	12.9	1.18	0.46	18	1.8	9.7	0.25	0.87
59	143	97	13.9	19.6	11.2	12.3	1.11	0.44	18	1.8	9.7	0.25	0.87
61	137	100	13.4	19.6	11.8	1 6	0.99	0.41	18	1.8	9.7	0.25	0.87
[illegible]	38	113	11.2	20.0	11.6	9.9	1.11	0.45	18	1.8	9.7	0.25	0.87
[illegible]	127	86	12.1	20	11.2	12.3	1.18	0.37	18	1.8	9.7	0.25	0.87
66	133	99	12.2	19.4	11.8	12.7	1.32	0.40	15	1.6	9.7	0.23	0.70
63	132	93	12	19.6	12.2	11.6	1.12	0.36	15	1.6	9.7	0.23	0.70
59	128	91	12.2	1 0	11.6	12.2	0.98	0.35	15	1.6	9.7	0.23	0.70

TABLE III

EXPERIMENT III

dat 1958	FOOD calories day	calories /kg body w	N mg./hr	S mg./hr	P mg./hr	K m eq./hr	C m.eq /hr	DETAILS	body wght kg
	Jan. 18								
18–20	3500	47 0	151	11 4	1.2	1.2	1.07		74 4
21–23									74.7
24–26								Jan. 4–26 +14.2 m.eq N Cl/hr	76 0
27–29									77 4
Febr									
30–1									76 4
2–4									76 4
5								Febr 5 NAD 25 mg.	76.6
6									76.55
7									76.5
5–7									
8									76.55
9									77.8
10									77.53
8–10									
11 13									77 05
14–16									77.5
17 19									77.5
20–22									77.9
23									78 1
24									78 7
25									78 6
23–25									
26				Febr 26 18.6				Febr 26 ANT 3 30 mg /os	78 6
27									78 8
28									79 15
26–28									
March									
1									79.1
2									79 2
3									79 55
1 3									

TABLE III

Experiment III

[illegible]	URINE: tot N mg/hr	urea mg N/hr	ammon. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m.eq/hr	Ca m.eq/hr	FAECES: N mg/hr	S mg/hr	P mg/hr	K m.eq./hr	Ca m.eq/hr
45	112	75	12.6	20.0	11.3	12.3	0.95	0.32	19	1.9	9.7	0.26	0.77
52	128	91	11.7	20.6	11.5	12.2	1.11	0.39	19	1.9	9.7	0.26	0.77
39	126	89	11.6	19.8	11.6	14.4	1.09	0.36	19	1.9	9.7	0.26	0.77
75	124	84	15.6	21.0	11.4	16.0	1.10	0.45	19	1.9	9.7	0.26	0.77
57	114	74	12.3	20.0	11.2	14.6	0.74	0.39	19	1.9	9.7	0.26	0.77
64	124	84	11.8	20.7	11.6	13.0	0.81	0.42	19	1.9	9.7	0.26	0.77
69	120	96	11.7	19.8	12.4	13.8	0.92	0.44	26	1.5	9.7	0.27	0.92
50	119	92	13.8	19.9	11.7	14.4	0.90	0.34	26	1.5	9.7	0.27	0.92
59	117	92	12.4	19.8	11.6	15.0	0.91	0.43	26	1.5	9.7	0.27	0.92
	119	93	12.6	19.8	11.9	14.4	0.91	0.40	26	1.5	9.7	0.27	0.92
9[illegible]	116	92	11.8	18.1	11.0	12.3	0.77	0.38	26	1.5	9.7	0.27	0.92
38	102	80	12.8	18.7	11.4	10.4	0.75	0.28	26	1.5	9.7	0.27	0.92
42	121	96	10.8	21.1	12.4	12.3	0.93	0.48	26	1.5	9.7	0.27	0.92
	113	90	11.8	19.3	11.6	11.7	0.82	0.38	26	1.5	9.7	0.27	0.92
57	120	92	14.2	19.4	11.6	12.5	1.15	0.40	26	1.5	9.7	0.27	0.92
63	95	73	11.4	19.8	11.2	14.0	1.10	0.45	26	1.5	9.7	0.27	0.92
44	104	80	11.8	20.9	11.2	11.6	0.98	0.29	26	1.5	9.7	0.27	0.92
54	130	96	12.1	20.2	11.7	12.5	0.98	0.39	26	1.5	9.7	0.27	0.92
47	116	82	11.2	21.0	11.1	13.1	0.94	0.34	26	1.5	9.7	0.27	0.92
30	107	74	11.8	20.5	12.1	13.4	0.82	0.24	26	1.5	9.7	0.27	0.92
56	136	108	13.5	20.7	12.8	14.2	0.83	0.38	26	1.5	9.7	0.27	0.92
	120	96	12.2	20.7	12.0	13.6	0.86	0.32	26	1.5	9.7	0.27	0.92
48	134	102	9.4	21.0	19.8	9.5	0.50	0.35	22	1.6	9.7	0.27	0.62
33	162	124	11.4	21.2	19.9	8.3	0.59	0.40	22	1.6	9.7	0.27	0.62
38	144	102	15.2	21.8	13.3	14.2	0.89	0.33	22	1.6	9.7	0.27	0.62
	147	109	12.0	21.3	17.9	10.7	0.66	0.36	22	1.6	9.7	0.27	0.6[illegible]
50	117	90	14.0	22.9	13.1	13.8	0.83	0.42	22	1.6	9.7	0.27	0.62
27	96	41	11.8	20.1	11.0	13.3	0.89	0.30	22	1.6	9.7	0.27	0.62
47	109	70	12.6	21.0	11.6	14.4	0.77	0.43	22	1.6	9.7	0.27	0.62
	107	70	12.8	21.3	11.9	13.8	0.83	0.39	22	1.6	9.7	0.27	0.62

TABLE IV

EXPERIMENT IV

	FOOD							DETAILS	
date 1958	*calories day*	*calories /kg body-w*	*N mg/hr*	*S mg./hr*	*P mg./hr*	*K m.eq./hr*	*Ca m.eq./hr*		*body-wgt kg*
Nov									
18									
19–21	2250	40 0	145	10.6	19 9	1[illegible]	1 16		56.2
22 4									55 7
25-27								Menstruation	55 85
28-30									55.5
Dec.									
1 3									55.5
4–6									55 6
7								NAPP 25 mg	55 4
8									55.2
9									55.4
7–9									
10									55 4
11									55 2
12									54 8
10-12									
13-15									54 9
16-18									54 5
19-21								Menstruation	54 4
22 24									54 1
25-27									54 5
28-30									54 8
Jan. 59									54 25
31 2									54 4
3								NAD [illegible]5 mg.	54 25
4									54 2
5									
3–5									54 3
6									54 25
7									54 1
8									
6–8									54 0
9–11									54 0
12									53.9
13–14									54 15
15–17									53 8
18–20									54.2
21 23									54 4
24–26									53 9
27 29									
Febr									53 3
30-1									53 4
2–4									53 5
5–7								Menstruation	53 6
8–12									53.6
13									53 5
14	400	7 5	0	0	0	0	0 5		53 6
15									53 4
16									53.3
17									53 2
18									53 0
19									

TABLE IV

EXPERIMENT IV

URINE									FAECES				
quant. ml /hr	tot N mg /hr	urea mg N /hr	amm. mg N /hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m eq. /hr	Ca m eq /hr	N mg /hr	S mg /hr	P mg /hr	K m eq /hr	Ca m eq /hr
128	528	480	21.5	20.4	37.4	34.2	3.5	0.44					
102	300	264	15.6	19.1	23.9	20.1	1.94	0.17	33	2.8	12.6	0.42	1.24
90	210	178	13.4	17.8	17.8	16.3	1.78	0.18	33	2.8	12.6	0.42	1.24
									33	2.8	12.6	0.42	1.24
94	180	158	10.8	16.4	14.6	15.4	1.51	0.23	33	2.8	12.6	0.42	1.24
95	164	139	11.4	16.6	13.3	13.2	1.31	0.22	33	2.8	12.6	0.42	1.24
108	160	132	9.7	17.0	[illegible]	11.4	1.53	0.245	33	2.8	12.6	0.42	1.24
100	166	146	8.9	16.9	11.6	8.9	1.24	0.22	31	3.5	12.6	0.47	1.38
94	158	136	9.9	17.1	14.6	11.5	0.80	0.225	31	3.5	[illegible]	0.47	1.38
100	160	132	9.9	17.4	14.3	11.2	1.07	0.265	31	3.5	12.6	0.47	1.38
98	161	134	9.6	17.1	13.5	10.5	1.04	0.24	31	3.5	12.6	0.47	1.38
95	168	137	11.0	16.9	12.9	10.9	1.40	0.24	31	3.5	12.6	0.47	1.38
97	154	126	10.3	16.7	12.7	8.4	1.27	0.28	31	3.5	12.6	0.47	1.38
88	149	129	11.0	17.0	12.8	16.0	[illegible]	0.245	31	3.5	12.6	0.47	1.38
93	157	128	10.7	16.9	12.8	11.8	1.30	0.255	31	3.5	12.6	0.47	1.38
91	151	119	9.7	16.7	12.2	13.0	1.60	0.19	31	3.5	12.6	0.47	1.38
97	175	149	10.8	17.3	12.6	12.2	1.81	0.21	31	3.5	12.6	0.47	1.38
									31	3.5	12.6	0.47	1.38
82	144	130	12.1	15.2	12.1	14.9	1.58	0.22	31	3.5	12.6	0.47	1.38
92	154	128	10.3	14.9	11.2	13.8	1.40	0.22	31	3.5	12.6	0.47	1.38
83	140	109	10.6	15.5	10.6	12.0	1.26	0.19	31	3.5	12.6	0.47	1.38
103	145	122	9.5	15.9	12.4	12.8	1.35	0.22	31	3.5	12.6	0.47	1.38
111	145	118	9.7	15.9	11.8	11.7	1.08	0.23	40	3.2	12.6	0.47	1.18
97	152	118	10.4	15.1	11.8	13.4	1.52	0.17	40	3.2	12.6	0.47	1.18
64	148	122	9.4	15.3	11.7	17.1	1.52	0.13	40	3.2	12.6	0.47	1.18
97	148	170	9.8	15.4	11.8	14.1	1.37	0.18	40	3.2	12.6	0.47	1.18
105	156	121	13.2	16.2	15.2	16.8	1.49	0.24	40	3.2	12.6	0.47	1.18
100	163	132	13.2	15.8	14.6	12.8	1.47	0.24	40	3.2	12.6	0.47	1.18
91	150	123	10.7	15.2	13.2	11.4	1.46	0.20	40	3.2	12.6	0.47	1.18
99	156	125	12.4	15.5	14.8	13.7	1.47	0.23	40	3.2	12.6	0.47	1.18
96	144	112	12.0	15.7	12.4	12.7	1.54	0.20	40	3.2	12.6	0.47	1.18
89	164	125	12.5	18.4	13.9	15.3	2.02	0.21	40	3.2	12.6	0.47	1.18
									40	3.2	12.6	0.47	1.18
93	15	112	12.2	14.9	9.7	12.4	1.65	0.25	40	3.2	12.6	0.47	1.18
85	141	114	11.1	14.0	10.2	12.2	1.55	0.24	40	3.2	12.6	0.47	1.18
77	130	105	7.6	14.7	10.6	11.2	1.63	0.23	40	3.2	12.6	0.47	1.18
105	136	106	7.4	14.8	10.0	11.8	1.69	0.28	40	3.2	12.6	0.47	1.18
99	154	118	9	14.7	10.2	12.4	1.56	0.25	40	3.2	12.6	0.47	1.18
99	151	120	7.9	17.4	10.8	14.6	1.97	0.22	40	3.2	12.6	0.47	1.18
95	149	116	9.6	16.6	12.5	13.0	1.67	0.26	40	3.2	12.6	0.47	1.18
89	160	125	9.6	15.6	11.6	13.2	1.60	0.23	40	3.2	12.6	0.47	1.18
									40	3.2	12.6	0.47	1.18
97	137	114	10.3	14.8	12.4	14.0	[illegible]	0.23	40	3.2	12.6	0.47	1.18
77	117	86	5.5	13.4	9.4	10.9	3.1	0.16	30	2.8	8.1	0.17	0.71
77	123	93	4.3	14.2	7.6	8.5	3.10	0.16	30	2.8	8.1	0.17	0.71
87	124	104	2.9	14.1	8.4	8.7	2.85	0.17	30	2.8	8.1	0.17	0.71
83	112	90	4.9	13.4	8.8	7.6	2.50	0.14	30	2.8	8.1	0.17	0.71
97	111	93	4.1	14.8	9.0	7.6	2.70	0.15	30	[illegible]	8.1	0.17	0.71
97	116	86	4.1	15.1	8.1	5.6	2.45	0.19	30	2.8	8.1	0.17	0.71

TABLE IV

EXPERIMENT IV

	FOOD							DETAILS	
date 1958	*calories day*	*calories /kg body-w*	*N mg./hr*	*S mg./hr*	*P mg./hr*	*K m eq./hr*	*Ca m.eq./hr*		*body weight kg*
Nov									
18									
19–21	2250	40.0	145	10 6	19 9	1.2	1 16		56.2
22 24									55 7
25–27								Menstruation	55.85
28–30									55.5
Dec.									
1 3									55.5
4–6									55 6
7								NAPP 25 mg.	55.4
8									55.[illegible]
9									55 4
7–9									
10									55.4
11									55.2
12									54 8
10–12									
13–15									54.9
16–18									54.5
19–21								Menstruation	54.4
22 24									54 1
25–27									54 5
28–30									54 8
Jan. '59									
31 2									54.25
3								NAD 25 mg	54 4
4									54.25
5									54 2
3–5									
6									54 3
7									54.25
8									54 1
6–8									
9–11									54 0
12									44 0
13–14									53.9
15–17									54 15
18–20									53 8
21 23									54.2
24–26									54 4
27 29									53 9
Febr									
30-1									53 3
2–4									53 4
5–7								Menstruation	53 5
8–12									53 6
13									53 6
14	400	7.5	0	0	0	2.0	0 5		53 5
15									53 6
16									53 4
17									53.3
18									53.[illegible]
19									53 0

TABLE IV

Experiment IV

URINE									FAECES				
quant ml/hr	tot N mg/hr	urea mg N/hr	ammon. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m.eq/hr	Ca m eq./hr	N mg/hr	S mg/hr	P mg/hr	K m eq/hr	Ca m eq/hr
103	100	81	4.4	14.4	7.9	2.0	2.20	0.20	30	2.8	8.1	0.17	0.71
98	91	73	3.6	13.8	8.3	3.4	2.33	0.12	30	2.8	8.1	0.17	0.71
103	92	74	2.9	14.8	5.6	6.4	2.80	0.13	30	2.8	8.1	0.17	0.71
95	86	65	4.2	16.1	5.4	3.9	2.15	0.09	30	2.8	8.1	0.17	0.71
98	86	66	4.1	14.7	7.2	5.0	2.35	0.13	30	2.8	8.1	0.17	0.71
106	95	73	4.5	14.2	8.0	5.7	3.12	0.13	30	2.8	8.1	0.17	0.71
92	95	76	5.1	13.8	21.7	3.4	2.60	0.14	30	2.8	8.1	0.17	0.71
78	82	65	3.3	15.6	10.3	2.8	2.40	0.08	30	2.8	8.1	0.17	0.71
94	88	69	3.2	14.9	7.5	4.2	3.10	0.11	30	2.8	8.1	0.17	0.71
93	95	65	2.6	14.7	5.9	5.4	2.70	0.13	30	2.8	8.1	0.17	0.71
96	92	64	4.0	13.8	6.1	6.1	2.55	0.15	30	2.8	8.1	0.17	0.71
100	96	70	4.2	14.6	7.1	7.3	2.72	0.12	30	2.8	8.1	0.17	0.71

TABLE IV

EXPERIMENT IV

	FOOD							DETAILS	
dat 1958	*calories day*	*calories /kg /body w*	*N mg /hr*	*S mg./h*	*P mg./hr*	*K m.eq./hr*	*Ca m eq /hr*		*body weight/ kg*
20	1200	22.8	0	0	0	2.0	0.5		52.6
21									52.3
22									52.3
23									51 7
24									51.4
25									51.[illegible]
				26 Febr					
26				7.2				ANT 3 30 mg.	90.8
27									50.5
28									90.9
March									
1									50.9
2									50.8
3									90 5

TABLE V A

Experiment

URINE									FAECES					BLOOD
quant. ml/hr	tot. N mg./hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m.eq/hr	Ca m.eq/hr	N mg/hr	S mg/hr	P mg/hr	K m.eq/hr	Ca m.eq./hr	urea mg/L
111	690	645	40.4	31.0	46.0	45.4	3.27	0.88	47	4.0	30.0	0.67	2.17	234
73	642	590	39.2	29.0	43.5	43.9	2.00	0.72	47	4.0	30.0	0.67	2.17	
71	610	570	38.3	30.1	41.1	48.9	1.86	0.76	47	4.0	30.0	0.67	2.17	287
71	590	544	37.6	29.6	47.0	52.0	1.66	0.59	47	3.0	30.0	0.67	2.17	
33	534	470	47.6	28.9	48.9	44.6	1.22	0.47	47	3.0	30.0	0.67	2.17	210
125	410	389	28.0	31.0	34.8	28.8	2.38	0.58	28.5	2.6	15.9	0.32	1.18	234
82	454	416	29.0	29.9	39.5	31.9	2.50	0.49	28.5	2.6	15.9	0.32	1.18	275
76	436	409	23.0	29.0	29.9	31.7	2.32	0.46	28.5	2.6	15.9	0.32	1.18	287
70	440	385	21.7	27.3	39.5	26.5	2.48	0.47	28.5	2.6	15.9	0.32	1.18	277
									28.5	2.6	15.9	0.32	1.18	291
48	470	395	31.5	28.9	46.7	42.0	1.97	0.44	50.9	4.9	36.0	0.66	2.46	208
74	510	445	23.9	28.5	43.5	37.0	2.50	0.44	50.9	4.9	36.0	0.66	2.46	
93	519	459	30.9	28.8	46.1	37.8	2.41	0.63	50.9	4.9	36.0	0.66	2.46	194
56	466	401	32.9	30.0	44.6	42.0	2.30	0.49	50.9	4.9	36.0	0.66	2.46	
74	441	394	30.0	30.5	42.9	45.6	2.00	0.63	50.9	4.9	36.0	0.66	2.46	
58	480	429	33.4	30.0	45.3	43.5	1.76	0.56	50.9	4.9	36.0	0.66	2.46	212
108	325	278	24.9	30.3	23.9	29.1	1.77	0.52	23.0	2.2	14.5	0.23	1.20	206
83	259	202	25.1	27.3	14.6	21.9	2.05	0.48	23.0	2.2	14.5	0.23	1.20	164
77	254	200	22.5	27.5	23.6	23.4	1.61	0.46	23.0	2.2	14.5	0.23	1.20	166
68	256	196	20.8	27.3	19.0	4.1	1.68	0.48	23.0	2.2	14.5	0.23	1.20	184
71	246	211	20.0	27.6	20.6	24.4	2.14	0.57	23.0	2.2	14.5	0.23	1.20	188
														202

TABLE V B

Experiment

URINE									FAECES					BLOOD
quant. ml/hr	tot. N mg/hr	urea mg N/hr	amm. mg/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m.eq/hr	C m.eq./hr	N mg/hr	S mg/hr	P mg/hr	K m.eq/hr	Ca m.eq/hr	urea mg/L
108	520	476	29.0	26.5	32.0	39.5	3.11	0.55	63	4.6	36.1	0.58	2.73	271
34	599	520	31.1	26.6	45.4	42.5	2.37	0.46	63	4.6	36.1	0.58	2.73	
73	581	530	32.0	76.6	42.5	47.0	2.45	0.55	63	4.6	36.1	0.58	2.73	351
75	580	529	32.0	26.6	44.2	54.5	2.11	0.57	63	4.6	36.1	0.58	2.73	
43.5	535	486	33.3	25.4	36.7	49.5	2.11	0.48	63	4.6	36.1	0.58	2.73	337
90	409	371	25.5	26.5	22.6	31.6	1.66	0.42	19.6	2.0	7.0	0.12	0.48	374
71	300	269	23.0	25.4	19.5	23.5	1.84	0.38	19.6	2.0	7.0	0.1	0.48	265
72	279	244	22.1	27.5	18.8	18.8	1.80	0.39	19.6	2.0	7.0	0.12	0.48	248
64	266	235	20.1	4.1	19.1	21.2	1.72	0.38	19.6	2.0	7.0	0.12	0.48	263
									19.6	2.0	7.0	0.12	0.48	299
65	490	430	27.9	24.6	35.0	45.5	2.27	0.51	40.4	3.5	4.7	0.37	1.92	273
[illegible]	445	409	26.0	25.0	34.1	41	2.51	0.54	40.4	3.5	24.7	0.37	1.92	
66	504	440	27.8	25.9	39.0	47.5	2.02	0.59	40.4	3.5	24.7	0.37	1.92	339
54	490	394	27.6	26.1	31.6	53.0	1.56	0.46	40.4	3.5	4.7	0.37	1.92	
43	475	18	30.2	25.4	38.2	49.5	1.53	0.35	40.4	3.5	24.7	0.37	1.92	
86	475	416	79.5	25.2	41.0	47.0	1.89	0.48	40.4	3.5	24.7	0.37	1.92	
109	319	276	20.8	4.6	4.3	26.3	2.58	0.38	40.4	3.5	24.7	0.37	1.92	238
90	340	298	21.5	25.5	79.1	24.9	[illegible]	0.36	25.0	0.74	5.6	0.15	0.44	238
83	371	332	19.6	26.9	26.9	[illegible]	2.47	0.30	25.0	0.74	5.6	0.15	0.44	259
									25.0	0.74	5.6	0.15	0.44	315
72	382	347	17.6	22.8	4.4	27.0	2.50	0.28	25.0	0.74	5.6	0.15	0.44	341
80	349	390	[illegible]	[illegible]	26.0	28.6	2.41	0.30	25.0	0.74	3.6	0.15	0.44	327

TABLE VA

EXPERIMENT V

date 1959	FOOD calories /day	calories /kg body w	N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	Ca m.eq./kg	body weight kg
April								
7	2980	38.9	533	38.0	73.6	1.8	2.9	76.6
8	2980	38.9	533	38.0	73.6	1.8	2.9	75.65
9	2980	38.9	533	38.0	73.6	1.8	2.9	75.45
10	2980	38.9	533	38.0	73.6	1.8	2.9	75.15
11	2980	38.9	533	38.0	71.6	1.8	2.9	75.05
12	400	5.3	0	0	0	2.0	0.45	75.55
13	400	5.3	0	0	0	2.0	0.45	73.4
14	400	5.3	0	0	0	2.0	0.45	72.8
15	400	5.3	0	0	0	2.0	0.45	72.25
16	400	5.3	0	0	0	2.0	0.45	71.9
22	2980	40.0	533	38.0	73.6	1.8	2.9	74.5
23	2980	40.0	533	38.0	73.6	1.8	2.9	74.9
24	2980	40.0	533	38.0	73.6	1.8	2.9	74.7
25	2980	40.0	533	38.0	73.6	1.8	2.9	73.85
26	2980	40.0	533	38.0	73.6	1.8	2.9	73.65
27	2980	40.0	533	38.0	73.6	1.8	9	73.65
28	1600	21.6	0	0	0	2.0	0.45	74.1
29	1600	21.6	0	0	0	2.0	0.45	72.8
30	1600	21.6	0	0	0	2.0	0.45	72.25
May								
1	1600	21.6	0	0	0	2.0	0.45	71.85
2	1600	21.6	0	0	0	2.0	0.45	71.6
3								

TABLE VB

EXPERIMENT V

date 1959	FOOD calories /day	calories /kg body w	N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	Ca m.eq./kg	body weight kg
April								
7	2530	38.9	531	37.4	73.1	1.8	2.9	65.0
8	2530	38.9	531	37.4	73.1	1.8	2.9	64.1
9	2530	38.9	531	37.4	73.1	1.8	2.9	64.15
10	2530	38.9	531	37.4	73.1	1.8	2.9	63.8
11	2530	38.9	531	37.4	73.1	1.8	2.9	63.6
12	1600	25.1	0	0	0	2.0	0.45	63.7
13	1600	25.1	0	0	0	2.0	0.45	63.0
14	1600	25.1	0	0	0	2.0	0.45	62.7
15	1600	25.1	0	0	0	2.0	0.45	62.4
16	1600	25.1	0	0	0	2.0	0.35	62.35
22	2530	40.0	531	37.4	73.1	1.8	2.9	63.45
23	2530	40.0	531	37.4	73.1	1.8	2.9	63.45
24	2530	40.0	531	37.4	73.1	1.8	2.9	63.45
25	2530	40.0	531	37.4	73.1	1.8	2.9	63.3
26	2530	40.0	531	37.4	73.1	1.8	2.9	62.95
27	2530	40.0	531	37.4	73.1	1.8	2.9	63.2
28	400	6.3	0	0	0	2.0	0.45	62.95
29	400	6.4	0	0	0	2.0	0.45	61.8
30	400	6.4	0	0	0	2.0	0.45	61.15
May								
1	400	6.4	0	0	0	2.0	0.45	60.55
2	400	6.4	0	0	0	2.0	0.45	60.35
3								

TABLE V A

[illegible]

				URINE							FAECES				BLOOD
[illegible] ml/hr	tot N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m eq./hr	Ca m eq./hr	N mg/hr	S mg/hr	P mg/hr	K m eq./hr	C m eq/hr	urea mg/l
111	650	615	40.4	31.0	46.0	45.4	1.27	0.88	47	4.0	30.0	0.67	2.17	234
73	642	590	39.2	29.0	43.5	43.9	2.00	0.72	47	4.0	30.0	0.67	2.17	
71	640	570	38.5	30.1	41.1	48.9	1.86	0.76	47	4.0	30.0	0.67	2.17	287
71	590	544	37.6	29.6	47.0	52.0	1.66	0.59	47	3.0	30.0	0.67	2.17	
51	531	470	47.0	28.9	48.9	44.6	1.22	0.47	47	3.0	30.0	0.67	2.17	210
125	410	349	28.0	31.0	34.8	28.8	2.38	0.58	28.5	2.6	15.9	0.32	1.18	234
82	456	416	29.0	29.9	39.5	31.9	2.50	0.49	28.5	2.6	15.9	0.32	1.18	275
76	436	409	23.0	29.0	29.9	31.7	2.32	0.46	28.5	2.6	15.9	0.32	1.18	287
74	440	385	21.7	27.3	39.5	26.5	2.48	0.47	28.5	2.6	15.9	0.32	1.18	277
									28.5	2.6	15.9	0.32	1.18	291
48	470	395	31.5	28.5	46.7	42.0	1.97	0.44	50.9	4.9	36.0	0.66	2.46	208
74	530	445	25.9	28.5	43.5	37.0	2.50	0.44	50.9	4.9	36.0	0.66	2.46	
93	519	459	30.9	28.8	46.1	37.0	2.41	0.63	50.9	4.9	36.0	0.66	2.46	196
56	466	401	32.9	30.0	44.6	42.0	2.30	0.49	50.9	4.9	36.0	0.66	2.46	
74	461	396	30.0	30.5	42.9	45.6	2.00	0.63	50.9	4.9	36.0	0.66	2.46	
58	480	429	33.4	30.0	45.3	43.5	1.76	0.56	50.9	4.9	36.0	0.66	2.46	212
101	325	278	24.9	30.3	23.9	29.1	1.77	0.52	23.0	2.2	14.5	0.23	1.20	206
83	258	202	25.1	27.3	14.6	21.9	2.05	0.48	23.0	2.2	14.5	[illegible]	1.20	164
77	236	200	22.5	27.5	23.6	23.4	1.61	0.46	23.0	2.2	14.5	0.23	1.20	166
48	236	196	20.8	27.3	19.0	24.1	1.68	0.48	23.0	2.2	14.5	0.23	1.20	184
71	246	211	20.0	27.6	20.6	24.4	2.14	0.57	23.0	2.2	14.5	0.23	1.20	188
														202

TABLE V B

[illegible]

				URINE							FAECES				BLOOD
[illegible] ml/hr	tot N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m eq/hr	Ca m eq/hr	N mg/hr	S mg/hr	P mg/hr	K m eq/hr	Ca m eq/hr	urea mg/L
100	520	476	29.0	26.5	32.0	39.5	3.11	0.55	63	4.6	36.1	0.58	2.73	771
74	599	520	31.1	26.6	45.4	42.5	2.37	0.46	63	4.6	36.1	0.58	2.73	
75	581	530	32.0	26.6	42.5	47.0	2.45	0.55	63	4.6	36.1	0.58	2.73	351
75	580	529	32.0	26.6	44.2	54.5	2.11	0.57	63	4.6	36.1	0.58	2.73	
43.5	533	486	33.3	25.4	36.7	49.5	2.11	0.48	63	4.6	36.1	0.58	2.73	337
90	409	371	25.5	26.5	22.6	31.6	1.66	0.42	19.6	2.0	7.0	0.12	0.48	374
71	300	269	23.0	25.4	19.5	23.5	1.84	0.38	19.6	2.0	7.0	0.12	0.48	265
72	279	244	22.1	27.5	18.8	18.8	1.80	0.39	19.6	2.0	7.0	0.12	0.48	248
64	266	235	20.1	24.1	19.1	21.2	1.72	0.38	19.6	2.0	7.0	0.12	0.48	263
									19.6	2.0	7.0	0.12	0.48	259
65	490	430	27.9	24.6	35.0	45.5	2.27	0.51	40.4	3.5	24.7	0.37	1.92	273
62	445	409	26.0	25.8	34.1	41.2	2.51	0.54	40.4	3.5	24.7	0.37	1.92	
66	504	440	27.8	25.9	39.0	47.5	2.02	0.59	40.4	3.5	24.7	0.37	1.9[illegible]	329
54	450	394	27.6	[illegible]4.1	31.6	53.0	1.56	0.46	40.4	3.5	4.7	0.37	1.92	
63	475	418	30.2	25.4	38.2	49.5	1.53	0.35	40.4	3.5	24.7	0.37	1.9[illegible]	
86	475	416	[illegible]9.5	25.2	41.0	47.0	1.89	0.68	40.4	3.5	24.7	0.37	1.92	238
109	319	276	20.8	24	24.3	26.3	2.50	0.38	25.0	0.74	5.6	0.15	0.44	[illegible]38
90	340	[illegible]98	21.5	25.5	[illegible]1	24.9	2.35	0.36	25.0	0.74	5.6	0.15	0.44	259
83	371	332	19.6	26.9	26.9	23.2	2.47	0.30	25.0	0.74	5.6	0.15	0.44	315
72	36[illegible]	347	12.6	[illegible]	24.4	27.0	2.30	0.28	25.0	0.74	5.6	0.15	0.44	341
80	369	330	[illegible]		26.0	28.6	2.61	0.30	25.0	0.74	5.6	0.15	0.44	327
														327

TABLE VI

date 1954	hour	FOOD calories /day	FOOD N mg./hr	FOOD S mg./hr	FOOD P mg./hr	FOOD K m.eq./hr	FOOD N m.eq./hr	body weight kg	URINE quant ml./hr	URINE total N mg./hr	URINE urea mg.N/hr	URINE amm mg.N/hr	URINE creatinine mg.N/hr	URINE S mg./hr	URINE P mg./hr	FAECES N mg./hr
Febr 11	18–21	2000	525	27.2	40 3	2.0	6.5	73 65	49	550		16.0	28.0	33 0	39 0	
	21–24								31	460		24 7	27.3	28.7	33 7	
12	0–3								31	480		26.3	27.3	31 7	36 0	
	3–6								41	580		27 7	29.3	37 7	33 7	
	6–9								78	620		13 0	30.0	33 3	24 7	
	9–12								117	670		11 3	27 0	35 0	19 3	
	12 15								101	630		15 0	29 0	31 7	23 3	
	15–18								100	580		8.3	29 0	30 7	28.0	
	18–21								71	560		11.3	29 0	33 0	22.3	
	21 24								72	540		11.3	29 7	35 3	16.0	
	0–24							73.65								
13	0–3								27	410		17 7	27.3	30 0	14 0	
	3–6								28	450		22.3	27 4	32.0	24.3	
	6–9								34	480		12.3	29 7	35.3	32.3	
	9–12								55	670		10.7	30.0	38 7	27.3	
	12 15								60	640		9.3	29.0	37 7	31 3	
	15–18								61	630		9.3	29 0	36.3	31.0	
	18–21								51	540		9.0	29 0	38.0	33.3	
	21 24								32	450		16.0	27 7	33.3	26.0	
	0–24							72.85								
14	0–3								27	470		19 0	27 0	33 3	25.3	
	3–6								31	530		20 7	28.0	36.0	19 7	
	6–9								42	600		12 0	28.3	40.3	31 0	
	9–12								73	680		9 7	28.3	38 7	27 0	
	12 15								72	680		8.7	27.3	36.7	28.0	
	15–18								78	620		9 3	27 0	28.3	38.0	
	18–21								65	610		9 0	29 0	28 7	36.3	
	21 24								72	540		12.0	28.0	28.3	20 0	
	0–24							73 0								
15	0–3								27	450		18.0	27 7	24 7	21.0	
	3–6								33	540		21 7	29 0	29.3	22.7	
	6–9								37	630		20.0	33 0	36 0	23 0	
	9–12								48	410		7 0	27.0	32 0	18.3	
	12 15								72	650		8 0	30 0	30 0	17 7	
	15–18								51	590		7 0	34.3	31 3	13 7	
	18–21								32	500		11.3	35.3	30.7	7 7	
21	18– 1	2000	525	27 2	40 3	2 0	6 5		137	550	90	26 9	27 9	34 3	18 0	
	21 4								[illegible]	440	90					

TABLE VI

22	[illegible]		33	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
	3		39	[illegible]	340	[illegible]	[illegible]		
	6-		44	400	340	[illegible]	23		
	9-12		74	400	350	23	23		
	12 15		2	400	350	21	25		2.8
	15-18		137	400	350	9 3	25		13.3
	18-21		47	390	340	15	27	21.3	5
	21 24		30	330	280	17 7	27 0	1 3	13 8
	0-.4	72 0		370	320	23 0	25 8	21.3	21 9	42.0
23	0-3		22	330	280	20 2	26 6	21 7	29 0	
	3-6		27	420	370	18 6	26 4	25.7	28 5	
	6-9		46	510	450	19.3	26 3	29.3	21 0	
	9-12		111	650	590	14.7	27 1	32.3	15.7	
	12 15		66	530	480	12 9	25.4	23 5	28 8	
	15-18		58	490	450	13.0	26 1	23 0	26.3	
	18-21		66	560	510	14 9	27 0	28.2	32.0	
	21 24		79	590	520	13.3	26.7	30 0	28 0	
	0-24	71 6		510	460	15.9	26 4	26.7	26.1	42 0
24	0-3		25	390	340	17 6	25.7	23.3	36.7	
	3-6		28	470	410	16.9	26 4	29 3	35.7	
	6-9		32	500	450	18 3	28 8	29.3	33.7	
	9-12		54	550	510	8.3	27 1	28 3	25 3	
	12-15		54	590	510	11 1	27.5	27.3	31 7	
	15-18		71	570	530	10 0	27.8	27 0	30.7	
	18-21		60	520	480	10.1	27.8	27.2	28 0	
	21 24		55	450	400	10.0	28 4	30.5	20 7	
	0-24	71.3		500	460	12 8	27.4	27.8	30.3	42 0
25	0-3		22	390	330	15.5	26 4	21 7	23.7	
	3-6		27	440	380	16 1	27.8	27.2	27 1	
	6-9		32	480	430	14 2	29 8	24.3	24.3	
	9-12		48	560	520	11 1	28.9	24.5	28.7	
	12 15		38	490	450	9.8	27.0	24 0	33 0	
	15 18		53	520	480	9 2	27 7	23 0	21 7	
	18-21		33	500	450	11 2	27 9	22 2	26 3	
	21 24		29	450	390	12.7	28 5	23 8	33.7	
	0- 4	71 3		480	430	12.2	27 9	23.8	27.3	42 0
26	0-3		22	420	370	14 2	26 5	22.2	32.7	
	3-6		38	560	510	16.5	27 8	28 8	35 7	
	6-6		91	610	550	12 8	27.0	32.3	28 0	
	9-12		120	630	590	10.1	28.0	28 8	20 0	
	13-15		152	540	500	10 6	27.8	26.3	18.0	
	15-18		33	450	400	11	27.2	22.7	31 0	
	18-21		31	440	400	10 5	28 3	23.7	33 0	
	21 24		26	410	360	10 6	27.8	25 0	4 0	
	0-24	71.1		510	460	12.1	27 6	26 2	27.8	42 0
27	0-3		25	420	370	13.8	27 6	29.0	27.3	
	3-6		28	480	430	16 3	27 9	39 3	30 7	

TABLE VI

		FOOD							URINE							FAECES
dat 1954	hour	calories /day	N mg./hr	S mg./hr	P mg./hr	K m.eq./hr	Na m.eq./hr	body weight kg	quant ml/hr	total N mg./h	urea mg.N/hr	amm. mg.N/hr	creatinine mg.N/hr	S mg./hr	P mg./hr	N mg./hr
Febr 11	18–21	2000	525	27.2	40.3	2.0	6.5	73.65	49	550		16.0	28.0	33.0	39.0	
	21–24								31	460		24.7	27.3	28.7	33.7	
12	0–3								31	480		26.3	27.3	31.7	36.0	
	3–6								41	580		27.7	29.3	37.7	33.7	
	6–9								78	620		13.0	30.0	33.3	24.7	
	9–12								117	670		11.3	27.0	35.0	19.3	
	12–15								101	630		15.0	29.0	31.7	23.3	
	15–18								100	580		8.3	29.0	30.7	28.0	
	18–21								71	560		11.3	29.0	33.0	22.3	
	21–24								72	540		11.3	29.7	35.3	16.0	
	0–24							73.65								
13	0–3								27	410		17.7	27.3	30.0	14.0	
	3–6								28	450		22.3	27.4	32.0	4.3	
	6–9								34	480		12.3	29.7	33.3	3[illegible]3	
	9–12								55	620		10.7	30.0	38.7	27.3	
	12–15								60	640		9.3	29.0	37.7	31.3	
	15–18								61	630		9.3	29.0	36.3	31.0	
	18–21								51	540		9.0	79.0	38.0	33.3	
	21–24								32	450		16.0	27.7	33.3	6.0	
	0–24							72.85								
14	0–3								27	470		19.0	27.0	33.3	25.3	
	3–6								31	530		20.7	28.0	36.0	19.7	
	6–9								42	600		12.0	28.3	40.3	31.0	
	9–12								73	680		9.7	28.3	38.7	27.0	
	12–15								72	680		8.7	27.3	36.7	28.0	
	15–18								78	620		9.3	27.0	28.3	38.0	
	18–21								65	610		9.0	29.0	28.7	36.3	
	21–24								72	540		12.0	28.0	28.3	20.0	
	0–24							73.0								
15	0–3								27	450		18.0	27.7	24.7	21.0	
	3–6								33	540		21.7	29.0	29.3	22.7	
	6–9								37	630		20.0	33.0	36.0	23.0	
	9–12								48	610		7.0	27.0	32.0	18.3	
	12–15								72	650		8.0	30.0	30.0	17.7	
	15–18								51	590		7.0	34.3	31.3	13.7	
	18–21								32	500		11.3	35.3	30.7	7.7	
21	18–21	2000	525	27.2	40.3	2.0	6.5		137	590	490	26.9	27.9	34.3	18.0	
	21–24								68	4[illegible]0	390					

TABLE VI

22	3		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	
	3-6		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
	6-9		[illegible]	400	[illegible]	30	23	23		
	9-12			400	50	23	23			
	12-15		24	400	380	21	25			
	15-18		137	400	330	5.3	25	1	2,3	
	18-21		47	390	340	15	27	21.3	9	
	21-24		30	330	280	17.7	27	19.3	1.8	
	0-24	72.0		370	330	23.0	25.8	21.3	21.9	42
23	0-3		22	330	280	20.2	26.6	21.7	29.0	
	3-6		27	420	370	18.6	26.4	25.7	28.5	
	6-9		46	510	450	19.3	26.3	29.3	21.0	
	9-12		111	650	590	14.7	27.1	32.3	15.7	
	12-15		64	530	480	12.9	25.4	23.5	28.0	
	15-18		58	490	450	13.0	26.1	23.0	26.3	
	18-21		66	560	510	14.9	27.0	28.2	32.0	
	21-24		79	590	520	13.3	26.7	30.0	28.0	
	0-24	71.4		510	460	15.9	26.4	26.7	26.1	42.0
24	0-3		25	390	340	17.6	25.7	23.3	36.7	
	3-6		28	470	410	16.9	26.4	29.3	35.7	
	6-9		32	500	450	18.3	28.8	29.3	33.7	
	9-12		54	550	510	8.3	27.1	28.3	25.3	
	12-15		54	550	510	11.1	27.5	27.3	31.7	
	15-18		71	570	530	10.0	27.8	27.0	30.7	
	18-21		60	520	480	10.1	27.8	27.2	28.0	
	21-24		55	450	400	10.0	28.4	30.5	20.7	
	0-24	71.3		500	460	1.8	27.4	27.8	30.3	42.0
25	0-3		22	390	330	15.5	26.4	21.7	23.7	
	3-6		27	440	380	16.1	27.8	27.2	27.1	
	6-9		32	480	430	14.2	28.8	24.3	4.3	
	9-12		48	560	520	11.1	28.9	24.5	28.7	
	12-15		38	490	450	9.8	27.0	24.0	33.0	
	15-18		53	520	480	9.2	27.7	23.0	21.7	
	18-21		33	500	450	11.2	27.9	22.2	26.3	
	21-24		29	450	390	12.7	28.5	23.8	33.7	
	0-24	71.3		480	430	12.2	27.9	23.8	27.3	42.0
26	0-3		22	420	370	14.2	26.5	22.2	32.7	
	3-6		38	560	510	16.5	27.8	28.8	35.7	
	6-6		91	610	550	12.8	27.0	32.3	28.0	
	9-12		120	630	590	10.1	28.0	28.8	20.0	
	12-15		152	540	500	10.6	27.8	26.3	18.0	
	15-18		33	430	400	11.7	27.2	22.7	31.0	
	18-21		31	440	400	10.5	28.3	23.7	33.0	
	21-24		26	410	360	10.6	27.8	25.0	24.0	
	0-24	71.1		510	460	12.1	27.6	26.2	27.8	42.0
27	0-3		25	420	370	13.8	27.6	29.0	27.3	
	3-6		28	480	430	16.3	27.9	39.3	30.7	

TABLE VI

dat 1954	hour	FOOD calories /day	N mg/hr	S mg/hr	P mg./hr	K m.eq./hr	N m eq/hr	body weight kg	URINE quant ml/hr	total N mg/hr	urea mg.N/hr	amm mg.N/hr	creatinine mg.N/hr	S mg/hr	P mg/hr	FAECES N mg./hr
	6–9								39	570	520	15 0	26.4	47.0	24.3	
	9–12								45	560	510	11 3	26.8	49.3	24 0	
	12 15								68	650	590	11 4	28 0	56 0	26.0	
	15–18								47	530	490	10.5	26.1	49 0	32.0	
	18–21								124	650	600	10.4	28 3	60 0	6.0	
	21 24								33	480	420	15 0	26 0	53 0	13 0	
	0– 4	2000	498	49 2	38 3	2.0	6.5	71.25		540	490	13.0	27.2	47.8	25 4	37 0
28	0–3								30	490	390	17 7	25.3	47.3	23.3	
	3–6								30	460	420	22.0	6.4	54.3	38 0	
	6–9								51	590	530	21 3	27 3	64 0	29 0	
	9–12								134	640	580	21 7	29 0	61.0	22.3	
	12 15								43	520	470	16.7	26.3	52.3	21 7	
	15–18								59	570	520	16.2	27.2	54 7	20.3	
	18–21								84	590	540	14 7	28.3	61 7	27.8	
	21 24								45	510	460	15.5	7.5	60.7	30.7	
	0– 4							71.2		550	490	18 2	27.2	57.0	26.6	37 0
March 1	0–3								33	480	420	22.0	25 7	49.3	35 0	
	3–6								38	550	490	21 6	25 7	58.7	32.7	
	6–9								39	560	500	26.3	6.8	61.3	20.7	
	9–12								99	620	550	23 7	28 0	62.3	19 7	
	12 15								103	630	570	17 3	26.5	61.3	16.5	
	15–18								54	490	430	17 3	25.5	51 7	15.8	
	18–21								125	610	560	19.2	28.6	63.3	26.3	
	21 24								54	470	420	18.9	28.0	56.7	14.0	
	0–24							71 0		550	490	20 8	26 9	58.1	22.6	37 0
2	0–3								33	490	430	23.9	27 1	51 3	25.5	
	3–6								32	480	420	8.3	27.5	59 3	24.2	
	6–9								33	520	450	29 7	28.2	62.0	22.3	
	9–12								70	650	600	16 6	28.5	66.7	19 8	
	12 15								61	600	550	14 9	7 7	60.7	16.8	
	15–18								33	470	420	20 8	27 4	51 7	23.7	
	18–21								109	670	610	21 4	9.3	66.7	35.3	
	21 24								47	510	450	19 0	28.7	56.7	25 7	
	0–24							70 65		550	490	21 8	27 9	59.3	24 2	37 0
3	0–3								26	460	400	22.2	26.3	48 0	3	
	3–6								30	510	490	26 2	28.0	42.7	22.7	
	6–								34	580	530	23 6	28 9	42.7	23 3	
									40	600	[illegible]					

TABLE VI

	2								47	580	30		26		23	
	15 1								62	600	540		28	34	22	
	1 21									30	790		29 8	34	22	
	21 24								1	530	490	1	27	30 3	19.	
		2000	423	27 2	40 3	2 0	6 5	70 55		360	900	17 7	27 9	38 2	22.2	40 0
4	0 3								27	440	400	15 3	26.3	27 0	16.3	
	3 6								29	490	440	18 5	27.3	31 0	24 7	
	6 9								34	550	500	20 0	28.4	35 0	22.2	
	9 12								42	560	510	13 0	28 0	32 0	19 3	
	12 15								44	580	530	12 6	27 7	33.7	17.0	
	15 18								57	600	550	12 3	28 7	29 0	24.3	
	18 1								120	660	610	10 0	27 7	24.3	37.3	
	21 24								74	540	480	9 3	26 6	[illegible]	21.2	
	0 4							70 6		560	900	13 9	27 3	31.8	22 8	40 0
5	0 3								25	430	370	15.1	24.7	24 7	22.0	
	3-6								31	520	470	20.7	26.3	30 7	30 8	
	6-9								41	610	560	17 5	27.6	33 0	24.3	
	9-12								54	580	530	13.9	28 0	32.7	13.5	
	12 15								83	590	530	11 7	27 8	29 0	22 0	
	15-18								112	640	600	8 6	27.3	22.7	26.5	
	18-21								105	610	570	8.9	27 4	29 8	28 3	
	21 24								87	580	540	7.3	26.5	31.0	31.3	
	0-24							70 25		570	510	13 0	26 6	29 2	24 8	40 0

TABLE VI

		FOOD							URINE							FAECES
dat 1954	*hour*	*calories /day*	*N mg./hr*	*S mg./hr*	*P mg./hr*	*K m.eq./hr*	*N m.eq./hr*	*body eight kg*	*quant ml./hr*	*total N mg./hr*	*urea mg.N/hr*	*amm. mg.N/hr*	*creatinine mg.N/hr*	*S mg./hr*	*P mg./hr*	*N mg./hr*
	6–9								39	570	520	15.0	26.4	47 0	24 3	
	9–12								45	560	510	11.3	26.8	49.3	24.0	
	12 15								68	650	590	11 4	28 0	56.0	26 0	
	15–18								47	530	490	10.5	26.1	49 0	32.0	
	18–21								124	650	600	10.4	28.3	60.0	26.0	
	21 24								33	480	420	15 0	26 0	53.0	13 0	
	0– 4	2000	498	49.2	38.3	2.0	6.5	71.25		540	490	13.0	27.2	47.8	25 4	37 0
28	0–3								30	490	390	17 7	25 3	47.3	23.3	
	3–6								30	460	420	22.0	26.4	54.3	38.0	
	6–9								51	590	530	21 3	27.3	64.0	[illegible]9.0	
	9–12								134	640	580	21 7	29 0	61 0	22.3	
	12 15								43	520	470	16.7	26.3	52.3	21 7	
	15–18								59	570	520	16.2	27.2	54 7	20.3	
	18–21								84	590	540	14.7	28.3	61 7	27.8	
	21 4								45	510	460	15.5	27 5	60.7	30.7	
	0–24							71.2		550	490	18.2	27.2	57 0	26.6	37 0
March 1	0–3								33	480	420	22.0	25 7	49 3	35.0	
	3–6								38	550	490	21 6	25 7	58.7	32.7	
	6–9								39	560	500	26.3	26.8	61.3	20.7	
	9–12								99	620	550	23 7	28.0	62.3	19 7	
	12 15								103	630	570	17.3	26.5	61 3	16.5	
	15–18								54	490	430	17.3	25.5	51 7	15.8	
	18–21								125	610	560	19.2	28.6	63.3	26.3	
	21 24								54	470	420	18 9	28 0	56.7	14 0	
	0–24							71 0		550	490	20.8	26 9	58.1	22.6	37 0
2	0–3								33	490	430	23 9	27 1	51.3	25.5	
	3–6								32	480	470	28.3	27.5	59 3	4.2	
	6–9								33	520	450	29 7	28.2	62.0	22.3	
	9–12								70	650	600	16.6	28 5	66.7	19 8	
	12–15								61	600	550	14 9	27 7	60.7	16.8	
	15–18								33	470	420	[illegible]0 8	27 4	51 7	23 7	
	18–21								109	670	610	21 4	29.3	66.7	35 3	
	21 4								47	510	450	19 0	28 7	56.7	25 7	
	0 24							70 65		550	490	21.8	27 9	59.3	24 1	37.0
3	0–3								26	460	400	[illegible]2.2	26.3	48 0	25 3	
	3–6								30	510	450	[illegible]6 2	28 0	42.7	22.7	
	6–9								34	580	520	23 6	28 9	42.7	23.3	
	9 12								40	600	550	[illegible]	[illegible]			

TABLE VII

[illegible]

			URINE				FAECES		
[illegible] ml/hr	tot. N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg/N/hr	S mg/hr	P mg/hr	N mg/hr	S mg/hr	P mg/hr
[illegible]	683	600	27.9	26.8	54.2	41.7	35	3.4	33.1
[illegible]	540	473	18.8	26.7	49.1	20.5	35	3.4	33.1
[illegible]	528	458	18.0	27.1	52.1	16.0	35	3.4	33.1
72	530	446	21.1	27.3	52.9	15.0	35	3.4	33.1
[illegible]	465	403	28.0	23.7	45.3	19.8			
27	470	403	31.0	27.3	48.7	28.5			
25	453	405	20.5	25.5	43.3	28.3			
[illegible]	593	530	29.3	29.4	55.3	10.5			
121	587	527	13.7	27.7	57.3	6.8			
177	593	540	11.7	28.3	55.7	11.3			
[illegible]	573	513	15.8	27.0	57.3	28.3			
51	400	423	22.5	27.0	55.3	25.3			
79	530	448	21.6	27.2	52.3	19.8	35	3.4	33.1
27	447	377	23.8	27.7	48.7	23.0			
23	383	321	23.8	25.5	33.7	32.0			
25	423	357	27.0	29.3	28.0	27.7			
[illegible]	440	377	25.2	30.0	34.3	14.7			
118	433	377	16.5	28.3	22.5	9.0			
122	310	440	12.0	27.8	49.7	13.5			
[illegible]	346	490	12.0	28.0	60.7	11.8			
[illegible]	610	357	14.7	27.7	79.3	12.3			
71	470	415	19.4	28.0	43.3	18.0	25	2.3	23.7
71	528	450	23.2	[illegible]	34.3	15.5	25	2.3	23.7
1	556	467	24.3	27.9	53.9	20.0	25	2.3	23.7
37	558	480	24.5	28.1	56.9	19.8	25	2.3	23.7
							25	2.3	23.7
40	613	537	27.3	26.9	64.7	16.7			
28	510	447	28.9	25.8	44.7	29.7			
33	513	443	31.3	27.4	37.7	79.7			
67	500	427	28.7	27.5	34.7	24.3			
125	540	433	25.3	25.9	39.3	8.7			
[illegible]	630	560	21.9	27.9	67.0	15.5			
105	643	600	11.0	27.2	77.3	10.3			
149	800	730	24.0	28.2	94.0	10.3			
85	594	[illegible]	24.8	[illegible]	57.4	16.9	25	2.3	23.7
57	683	610	25.3	28.3	73.0	19.7			
47	600	540	28.7	25.3	63.0	24.3			
[illegible]	590	520	34.7	26.0	49.3	30.2			
64	530	463	29.7	23.3	52.7	19.3			
73	617	540	18.5	27.3	64.0	10.9			
53	560	527	15.1	26.3	46.7	14.5			
45	528	460	29	26.2	57.0	16.0			
40	[illegible]	10	[illegible]	[illegible]	60.7	23.0			
53	587	520	26.2	26.3	60.4	19.7	22	1.9	18.0
29	490	420	1.2	25	51.0	19.2			
[illegible]	507	77	[illegible]	25.8	52.7	20.3			
[illegible]	3	470	32.3	25.9	55.0	17.7			
2	551	490	27	27.0	50.3	14.0			
148	627	10	4	[illegible]	61.0	9.7			
44	650	560	17.3	28.0	61.3	10.0			
97	590	540	11.8	[illegible]	53.7	13.0			
74	54	490	12	[illegible]	52.3	13.8			

TABLE VII

EXPERIMENT VII

date 1954	hour	FOOD calories /day	N mg./hr	S mg./hr	P mg./hr	K m.eq./hr	N m.eq./hr	DETAILS	body-weight kg
March									
14		2144	415	41 0	31.8	2.0	6.5	Food equally divided	72.4
15									71 75
16									71 4
17									70 9
18	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								
	0–24								70 85
19	0–3							Protein and carbohydrate separated	
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								70 8
	0–24								70.35
20									70 25
21									70 0
22									70 05
23									
24	0–3								
	3–6								
	6–9								
	9–1								
	12 15								
	15–18								
	18–21								
	21 24								69 9
	0–24								
25	0–3							Food equally divided	
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18 21								
	21 24								
	0–24								
26	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								

TABLE VII

[illegible]MENT VII

quant ml/hr	tot N mg/hr	URINE urea mg N/hr	ammon mg N/hr	creatinine mg/N/hr	S mg/hr	P mg/hr	FAECES N mg/hr	S mg/hr	P mg/hr
[illegible]	643	600	27.9	26.8	54.2	41.7	35	3.4	33.1
[illegible]	540	475	18.8	26.7	49.1	20.5	35	3.4	33.1
[illegible]	528	458	18.0	27.1	52.1	16.0	35	3.4	33.1
72	530	446	21.1	27.3	52.9	15.0	35	3.4	33.1
79	465	403	28.0	25.7	45.3	19.8			
27	470	403	31.0	27.3	48.7	28.5			
25	453	405	20.5	25.5	43.3	28.3			
[illegible]	593	530	29.3	29.4	55.3	10.5			
122	587	527	13.7	27.7	57.3	6.8			
131	593	540	11.7	28.3	55.7	11.3			
[illegible]	573	513	15.8	27.0	57.3	28.3			
51	500	423	22.5	27.0	55.3	25.3			
79	530	468	21.6	27.2	52.3	19.8	35	3.4	33.1
27	447	377	23.8	27.7	48.7	23.0			
23	383	321	23.8	25.5	33.7	32.0			
25	423	357	27.0	29.3	28.0	27.7			
[illegible]	440	377	25.2	30.0	34.3	14.7			
[illegible]	433	377	16.5	28.3	22.5	9.0			
122	510	460	12.0	27.8	49.7	13.5			
96	546	490	12.0	28.0	60.7	11.8			
[illegible]	610	557	14.7	27.7	79.3	12.3			
71	470	415	19.4	28.0	43.3	18.0	25	2.3	23.7
71	528	490	23.2	27.2	54.3	15.5	25	2.3	23.7
71	556	467	24.3	27.9	53.9	20.0	25	2.3	23.7
57	550	480	24.5	28.1	56.9	19.8	25	23.	23.7
							25	2.3	23.7
[illegible]	613	537	27.3	26.9	64.7	16.7			
25	510	447	28.9	25.8	44.7	29.7			
31	513	443	31.3	27.4	37.7	29.7			
97	500	427	28.7	27.5	34.7	24.3			
125	540	453	25.3	25.9	39.3	8.7			
99	630	560	21.9	27.9	67.0	15.5			
105	643	600	11.0	77.2	77.3	10.3			
140	800	730	4.0	28.2	94.0	10.3			
85	594	525	4.8	27.1	57.4	16.9	25	2.3	23.7
57	683	610	25.3	26.3	73.0	19.7			
47	600	540	28.7	23.3	63.0	24.3			
36	590	520	34.7	28.0	99.3	30.2			
[illegible]	30	463	29.7	25.3	52.7	19.3			
73	17	540	18.5	27.3	64.0	10.5			
53	560	527	1 1	26.3	56.7	14.5			
45	520	460	29.9	26.2	52.0	16.0			
40	597	510	77.7	79.0	60.7	23.0			
53	587	20	26.2	26.3	60.4	19.7	22	1.9	18.0
79	490	420	1.2	25.2	51.0	19.2			
27	507	437	28.3	25.8	52.7	20.3			
79	511	470	3. 3	25.9	55.0	17.7			
42	53	490	27.3	27.0	50.3	14.0			
144	77	30	4.5	77.0	64.0	9.7			
146	650	560	17.3	28.0	61.3	10.0			
97	580	540	11.8	27.0	53.7	13.0			
74	543	490	1_.2	26.3	52.3	13.8			

TABLE VII

EXPERIMENT VII

date 1954	hour	FOOD calories /day	N mg./hr	S mg./hr	P mg./hr	K m.eq./hr	N m.eq./hr	DETAILS	body weight kg
	0–24								69.4
27									69.35
28									69
29	0–3								
	3–6								
	6–9								
	9–12								
	12–15								
	15–18								
	18–21								
	21–24								
	0–24								69.2
30	0–3							Growth hormone 100 mg.	
	3–6								
	6–9								
	9–12								
	12–15								
	15–18								
	18–21								
	21–24								
	0–24								68.8
31									68.3
April 1									68.05

TABLE VII

Experiment VII

			URINE				FAECES		
quant. ml/hr	tot. N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg/N/hr	S mg/hr	P mg/hr	N mg/hr	S mg/hr	P mg/hr
74	599	492	21.9	26.5	54.6	14.7	22	1.9	18.0
74	508	444	22.1	26.7	52.5	15.9	22	1.9	18.0
64	490	430	27.5	26.3	53.3	14.2	22	1.9	18.0
34	420	360	20.0	27.0	44.7	12.5			
24	420	380	27.7	26.2	47.3	15.5			
22	393	333	29.5	23.3	42.7	13.7			
33	523	450	34.0	29.9	54.0	16.5			
85	503	460	25.7	26.3	51.7	7.7			
102	497	447	15.7	26.0	48.3	8.8			
123	513	463	12.8	27.4	51.0	17.3			
148	520	470	12.5	26.5	52.7	16.7			
71	474	418	22.2	26.8	49.3	13.6	22	1.9	18.0
44	457	393	20.3	26.8	52.0	25.3			
35	440	387	25.3	25.4	49.0	18.3			
27	443	380	36.3	26.3	49.3	16.3			
29	493	423	30.8	29.3	48.7	9.7			
87	513	440	22.3	27.3	49.0	8.2			
165	540	407	15.0	28.7	50.7	8.3			
130	497	437	19.3	27.4	48.0	16.5			
133	490	453	16.8	27.5	55.3	21.7			
81	484	425	23.3	27.3	50.8	15.5	22	1.9	18.0
68	500	430	19.7	26.3	54.1	19.2	22	1.9	18.0
65	500	437	20.8	26.4	54.0	16.3	22	1.9	18.0

TABLE VII

EXPERIMENT VII

		FOOD						DETAILS	
date 1954	*hour*	*calories /day*	*N mg./hr*	*S mg./h*	*P mg./hr*	*K m.eq./hr*	*N m.eq./hr*		*body weight kg*
	0–24								69.4
27									69.35
28									69.2
29	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								
	0–24								69._
30	0–3							Growth hormone 100 mg.	
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								
	0–24								68.8
31									68.3
April 1									68.05

TABLE VIII

Experiment VIII

	URINE						FAECES		
quant. ml/hr	tot. N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	N mg/hr	S mg/hr	P mg/hr
93	580	510	22	29	35	33	23	2.4	8.9
90	700	620	26	28	38	38	23	2.4	8.9
96	770	690	34	28	38	41	23	2.4	8.9
71	680	610	35	28	37	41	23	2.4	8.9
70	680	610	39	29	38	45	23	2.4	8.9
50	560		36	29	30	38			
41	600		48	28	34	41			
82	670		50	28	35	32			
72	600		48	27	34	39			
87	700		44	31	32	43			
73	650		47	29	33	55			
76	660		45	29	32	38			
115	600		43	30	35	39			
	650	560			33	41	23	2.4	8.9
88	590		37	26	32	35			
45	470		37	26	27	34			
78	480		45	26	23	31			
92	590		59	34	29	41			
95	560		74	30	30	54			
95	600		77	29	36	55			
129	730		83	31	47	54			
73	780		74	29	47	42			
	590	480			34	39	23	2.4	8.9
28	340	270	26	30	23	30			
53	420	360	12	28	25	8			
74	480	420	18	25	27	14			
62	490	430	15	27	27	17			
82	500		19	26	26	23			
47	470		26	25	26	18			
144	610		20	28	29	8			
86	510		5	27	26	4			
86	530		10	27	26	15			
100	520		17	24	24	32			
104	570		13	[illegible]	28	39			
45	400		10	25	25	23			
	520	470			26	20			
41	420		12	21	19	13			
63	550		27	31	[illegible]	19			
120	550		18	27	25	10			
88	490		12	29	26	4			
64	500		15	28	26	7			
117	460		13	28	31	14			
70	520		15	[illegible]	26	4			
35	390		16	28	18	28			
	500	490			26	15			
49	490	440	14	28	[illegible]	15			
[illegible]	460	390	20	[illegible]	29	11			

TABLE VIII

EXPERIMENT VIII

date 1954	hour	FOOD calorie /day	N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	N m.eq./kg	DETAILS	body weight kg
April 21		2400	437	22.7	33.6	2.0	6.5	N carbohydrat	73 8
22									72.9
23									72.7
24									72.0
25									71 8
26	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21 24								71 5
	0–24								
27	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	21–24								71 3
	0–24								70.5
28								155 g butter ↓	71.3
29		2400	437	22.7	33 6	‿0	6.5	304 g sugar	71.5
30									
May 1									71 7
									71 45
2									
3	0–3								
	3–6								
	6–9								
	9–12								
	12 15								
	15–18								
	18–21								
	1 24								71 2
	0– 4							Androstadienolone 50 mg	
4	0–3								
	3–6								
	6–9								
	9–12							Androstadienolone 50 mg	
	12 15								
	15–18								
	18–21								
	21 24								71 2
	0–24							Androstadienolone 2 50 mg.	71.3
5								Androstadienolone 2 50 mg.	71 4
6									

TABLE VIII

Experiment VIII

			URINE				FAECES		
vol. ml/hr	tot N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg/N/hr	S mg/hr	P mg/hr	N mg/hr	S mg/hr	P mg/hr
93	580	510	22	29	35	33	23	2.4	8.9
90	700	620	26	28	38	38	23	2.4	8.9
96	770	690	34	28	38	41	23	2.4	8.9
71	680	610	35	28	37	41	23	2.4	8.9
78	680	610	39	29	38	45	23	4	8.9
58	560		36	29	30	38			
41	600		48	28	34	41			
82	670		50	28	35	32			
73	680		48	27	34	39			
87	700		44	31	32	43			
73	650		47	29	33	55			
76	660		45	29	32	38			
113	680		43	30	35	39			
	650	560			33	41	23	2.4	8.9
88	550		37	26	32	35			
45	470		37	26	27	34			
78	480		45	26	23	31			
92	590		59	34	29	41			
95	560		74	30	30	54			
95	600		77	29	36	55			
175	730		83	31	47	54			
73	700		94	29	47	42			
	590	480			34	39	23	2.4	8.9
23	340	270	26	30	23	30			
35	420	360	12	28	25	8			
74	480	420	18	25	27	14			
62	490	430	15	27	27	17			
83	500		19	24	26	23			
42	470		26	25	26	18			
144	610		20	28	29	8			
86	510		5	27	26	4			
86	530		10	27	26	15			
100	20		17	4	24	32			
104	570		13	77	28	39			
45	400		10	23	25	23			
	20	470			26	20			
41	420		12	21	19	13			
65	550		27	31	[illegible]	19			
120	550		18	27	25	10			
88	490		12	[illegible]	26	4			
64	500		15	28	26	7			
117	560		15	28	31	14			
70	520		15	[illegible]	26	24			
38	390		16	28	18	28			
	500	430			26	15			
49	480	440	14	26	28	13			
[illegible]	440	390	20	[illegible]	29	11			

TABLE IX

Experiment IX

date 1954	FOOD calories /day	N mg./hr	S mg./hr	P mg./hr	K m.eq./hr	Na m.eq./hr	DETAILS	body-weight kg
May								
25	2400	109	5.7	8.4	2.0	6.5	Food equally divided	70.8
26								69.9
27								69.2
28								69.1
29								69.2
30								69.35
31								69.2
June								
1							Protein and carbohydrate separately	69.1
2								69.05
3								69.0
4								69.05
5								68.9
6							Food equally divided	68.8
7								68.85
8								68.7
9								68.6
10								68.5
11							Protein and fat separately	68.45
12								68.3
13								68.2
14								68.1
15								68.0
16							Food equally divided	67.85
17								67.8
18								67.8
19								67.65
20								67.6
21							Protein and carbohydrat separately	67.7
22								67.6
23								67.7
24								67.5
25							Food equally divided	67.4
26								67.55
27								67.4
28								67.3
29								67.15
30								67.15
July								
1								67.1
2							Androstadienolon 3 × 75 mg.	67.05
3							Androstadienolon 3 × 75 mg.	67.0
4								66.7
5								66.85
6								66.9
7								66.8
8								66.85
9								66.95
10								66.9
11								66.8
12								66.9

TABLE IX

[illegible]

[illegible]	URINE								FAECES					BLOOD
[illegible]	Total N mg /hr	Urea mg N /hr	Ammon. mg N /hr	Creatinine mg N /hr	S mg /hr	P mg /hr	K m eq /hr	Na m eq. /hr	N mg /hr	S mg /hr	P mg /hr	K m eq /hr	Na m eq. /hr	Urea mg /L
[illegible]	310	270	9 8	26 0	18 3	22 5	2 4	9.3	32	2.8	18 5	0.3	0.8	
77	240	190	11.3	28 0	14 8	19.5	1 7	8 2	32	2 8	18.5	0.3	0 8	
[illegible]	200	160	11.1	25 0	13 0	14.6	1.5	5 5	32	2.8	18.5	0.3	0 8	175
[illegible]	170	130	10 6	22 0	11 5	11.5	1.2	3 8	32	2.8	18 5	0.3	0.8	
[illegible]	200	160	11 1	26 0	13.5	11.9	1.6	5 3	32	2 8	18.5	0.3	0 8	
[illegible]	180	140	11.0	24 0	13.0	12.3	1 6	5 6	32	2 8	18 5	0.3	0 8	
[illegible]	200	150	11.5	27.0	14 4	15.0	0	7.0	32	2.8	18.5	0.3	0 8	
[illegible]	220	170	13 5	25.0	13.7	15.7	1.9	4 7	31	2 6	5.0	0 4	0.9	190
[illegible]	240	180	13 3	26 0	13.1	18.5	2 3	5.3	31	2 6	5 0	0 4	0 9	
[illegible]	200	150	13.2	27 0	13 3	14.3	1.9	4 2	31	2.6	5.0	0 4	0 9	
[illegible]	200	150	13 7	26 0	13.7	13.3	1.8	5 1	31	2.6	5 0	0 4	0.9	125
71	200	150	16 9	25 0	12 7	13.7	1 9	5 8	31	2 6	5.0	0 4	0.9	
[illegible]	180	130	12.7	27 0	12 5	12 5	1 6	6 0	22	1 7	4 5	0 2	0.3	132
74	160	110	13 5	25 0	11.9	10 0	1.3	6 4	22	1 7	4 5	0 2	0 3	
[illegible]	160	110	10 0	25 0	11 9	11.5	1 7	6.2	22	1 7	4.5	0 2	0 3	106
[illegible]	180	130	10 0	25 0	11.9	11 3	1 8	5 7	22	1 7	4 5	0.2	0 3	
[illegible]	190	130	10 2	25.0	11 4	12 0	1 7	6 0	22	1 7	4.5	0 2	0.3	
[illegible]	180	130	10.8	25 0	11 5	12 5	1 7	4.9	15	1.3	2.6	0.2	0 1	123
[illegible]	170	120	10 5	25 0	11 4	12 4	2 0	5 2	15	1.3	2 6	0.2	0.1	
72	170	130	11 0	25 0	11.3	15 9	2.1	6 0	15	1.3	2 6	0.2	0 1	136
[illegible]	180	140	9 9	25 0	11 6	17 7	2 2	7 1	15	1 3	2.6	0 2	0.1	
[illegible]	180	130	9.8	23 0	11 0	16 1	1 9	5.7	15	1.3	2 6	0 2	0.1	1.3
[illegible]	170	130	10 7	25 0	10.8	16.4	1 7	6 8	16	1 35	2.7	0 2	0.1	
72	160	120	9 1	24 0	10 7	15 9	1 7	6 0	16	1 35	2.7	0.2	0 1	
[illegible]	170	130	10 5	26 0	11 3	15 0	1.7	7 2	16	1.35	2.7	0 2	0.1	
[illegible]	150	110	10 3	25 0	10 4	14 4	1 4	5 0	16	1.35	2.7	0.2	0 1	
[illegible]	170	120	9.2	26 0	10 6	15 9	1 7	5.8	16	1 35	2.7	0 2	0.1	134
[illegible]	190	150	11 9	24 0	11 0	15 9	2 0	4 7	21	1 7	3 8	0 4	0 3	
[illegible]	200	150	11 6	26 0	11 8	17 0	2 0	4 9	21	1 7	3.8	0 4	0.3	136
[illegible]	180	140	11 0	25 0	11 4	16.9	1.8	5.6	21	1 7	3 8	0 4	0.3	
[illegible]	170	130	11 0	25.0	11 3	15 7	1 8	5 9	21	1 7	3 8	0 4	0 3	
[illegible]	170	130	11 3	25 0	10 7	15 0	1 6	5 6	21	1 7	3 8	0 4	0 3	
[illegible]	150	110	12 1	25 0	9 6	14.2	1 4	5 4	15	1 3	3 1	0 2	0 1	
[illegible]	150	110	8 8	25 0	10 0	14 2	1 7	6 6	15	1.3	3 1	0 2	0 1	128
74	140	100	7 9	24 0	9 4	12 9	1 8	6 6	15	1 3	3 1	0 2	0.1	
75	150	110	9 0	23 0	9 6	14 0	1 6	6.3	15	1.3	3.1	0 2	0.1	[illegible]
71	190	110	8 7	4 0	9 8	12 7	1 7	5 6	15	1 3	3.1	0.2	0.1	
[illegible]	160	120	8 2	25 0	9 8	16 9	1 8	7 0	15	1.3	3 1	0 2	0.1	
[illegible]	140	100	8 4	25 0	9 2	11 7	1 7	5 9	15	1 3	3 1	0 2	0 1	116
[illegible]	140	100	7 8	25 0	9 0	10 9	1 4	6 8	19	1 6	4.1	0 3	0.2	81
65	150	110	11 0	26 0	11 3	6 0	1 2	6 0	19	1 6	4 1	0 3	0 2	
[illegible]	150	100	15 7	25 0	10 0	9 1	0 8	4 0	19	1 6	4 1	0 3	0.2	
79	170	120	10	26.0	11 1	9 3	1	6.3	19	1 6	4 1	0 3	0.2	
63	140	100	11 4	25 0	10 6	12 1	1.2	5 1	19	1 6	4 1	0 3	0.2	121
80	130	90	9 0	2 0	9 8	11 2	1.5	6 0	19	1.6	4 1	0.3	0.2	
75	140	100	7 1	25 0	9 4	10 3	1 6	7 3	19	1 6	4 1	0 3	0.2	
[illegible]	120	80	7 2	24 0	8 8	10 8	1 6	5.9	19	1.6	4 1	0 3	0.2	
57	140	100	6 8	25 0	8 9	10.2	1.9	5 6	19	1 6	4 1	0.3	0 2	
79	150	100	9.2	25 0	7 8	11 4	2.0	5 8	19	1.6	4 1	0.3	0.2	97

TABLE X

Experiment x

date 1955	calories /day	FOOD N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	N m.eq./kg	MED	body-weight kg
Jan								
6	2370	436	22.8	33 6	2.0	6.5		62.8
7								6[illegible].6
8								62.3
9								62.1
10								61.9
11							MAD 90 mg.	62.0
12							MAD 90 mg.	62 1
13							MAD 90 mg.	62.4
14							MAD 90 mg.	62 2
15								62.0
16								62.1
17								62.1
18								62 0
19								62.0
20								61 7
21								61.6
22								61 7
23								61.65
24								61.8
25								61 7

TABLE X

Experiment	URINE								FAECES					BLOOD
quant. ml/hr	total N mg /hr	urea mg N /hr	amm. mg N /hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m eq. /hr	Na m eq /hr	N mg /hr	S mg /hr	P mg /hr	K m eq. /hr	N m.eq. /hr	urea mg /L
69	500	441	14.4	23.5	29.2	34.1	2.3	7.4	21	2.7	14.4	0.5	0.2	
63	496	435	11.7	24.5	27.6	29.4	2.6	7.7	21	2.7	14.4	0.5	0.2	332
63	502	455	12.6	23.5	30.3	28.2	2.5	6.4	21	2.7	14.4	0.5	0.2	
57	501	445	12.6	24.0	30.5	25.9	2.2	5.4	21	2.7	14.4	0.5	0.2	
43	466	435	12.4	24.4	26.5	22.1	1.9	5.4	21	2.7	14.4	0.5	0.2	348
41	469	430	12.2	25.1	26.5	18.4	1.8	5.5	24	2.9	16.8	0.5	0.2	
38	431	411	12.8	25.2	26.1	14.6	1.5	7.0	24	2.9	16.8	0.5	0.2	
49	433	415	14.7	26.4	27.0	21.4	1.4	7.1	24	2.9	16.8	0.5	0.2	272
47	415	384	12.9	24.0	26.9	22.2	1.6	5.8	24	2.9	16.8	0.5	0.2	
48	420	384	19.8	22.6	27.4	22.2	1.9	6.6	54	4.7	12.7	0.6	0.3	
50	427	390	12.6	22.6	26.0	23.2	1.9	6.5	54	4.7	12.7	0.6	0.3	
35	389	360	12.4	22.7	25.2	19.6	1.8	5.3	54	4.7	12.7	0.6	0.3	
35	386	338	18.3	24.2	25.0	16.3	1.3	4.6	54	4.7	12.7	0.6	0.3	
26	386	336	18.7	24.5	22.5	13.4	1.2	5.0	54	4.7	12.7	0.6	0.3	
34	375	337	15.5	23.9	22.0	12.4	1.4	5.1	54	4.7	12.7	0.6	0.3	
39	384	341	12.0	22.9	20.6	14.4	1.3	5.2	54	4.7	12.7	0.6	0.3	
48	378	332	9.8	22.6	17.6	16.8	1.3	5.4	54	4.7	12.7	0.6	0.3	
47	398	326	8.9	22.8	15.8	24.0	1.6	6.5	54	4.7	12.7	0.6	0.3	
43	350	308	9.0	22.9	16.0	22.6	1.6	6.1	54	4.7	12.7	0.6	0.3	
54	395	365	7.6	23.1	19.0	24.2	1.9	8.0	54	4.7	12.7	0.6	0.3	265

TABLE XI

EXPERIMENT XI

date 1955	FOOD calories /day	N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	N m.eq./kg	MED	body-weight kg
Jan.								
6	100	109	5 7	8 4	2.0	6.5		56.4
7								56 1
8								55.7
9								55.5
10								55 6
11							MAD 90 mg.	55.6
12							MAD 90 mg	55 6
13							MAD 90 mg.	55.7
14							MAD 90 mg.	55.45
15								55 6
16								55 5
17								55.4
18								55.2
18								55 15
20								55 1
21								55 15
22								54.85
23								54.95
24								55.0
25								55.05

TABLE XII

EXPERIMENT XII

date 1955	FOOD calorie /day	N mg./kg	S mg./kg	P mg./kg	K m.eq./kg	N m.eq./kg	MED	body-weight kg
March								
25	2690	393	19.3	33 3	2.0	6.5		72.7
26								72 5
27								71 6
28								71.8
29								72 0
30								71 9
31								71 7
April 1								71.8
2							TP 30 mg. + Hyaluronidase 100 u.	71 5
3								71 4
4								71 15
5								71.9
6								72.2
7								72.5
8								71 9
9								71.55
10							Hyaluronidase 100 u.	71.05
11								71.0
12								70.8
13								70.8

TABLE XI

Experiment XI

	URINE								FAECES					BLOOD
quant ml/hr	total N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m eq/hr	Na m eq/hr	N mg/hr	S mg/hr	P mg/hr	K m eq/hr	N m eq/hr	urea mg/L
79	263	240	6.7	19.9	14.7	3.4	2.1	8.1	20	1.6	6.0	0.6	0.6	222
58	193	170	7.8	20.5	12.6	8.9	2.2	5.2	20	1.6	6.0	0.6	0.6	
80	221	196	3.4	20.6	12.9	10.5	2.5	7.2	20	1.6	6.0	0.6	0.6	
79	198	167	6.5	20.2	13.4	10.4	2.1	6.0	20	1.6	6.0	0.6	0.6	
50	174	154	8.8	19.8	12.2	10.4	1.8	5.0	20	1.6	6.0	0.6	0.6	
50	168	140	7.9	21.2	12.0	7.5	1.6	5.2	20	1.7	6.7	0.5	0.5	192
54	156	126	7.9	20.0	11.2	6.5	1.7	5.3	20	1.7	6.7	0.5	0.5	
68	154	132	7.3	20.4	11.3	7.5	1.8	6.0	20	1.7	6.7	0.5	0.5	
55	143	118	6.8	19.5	11.0	5.0	1.5	6.1	20	1.7	6.7	0.5	0.5	165
64	149	120	7.7	19.8	11.5	6.1	1.8	6.6	17	1.5	5.5	0.5	0.5	
[illegible]	145	116	8.0	19.5	11.2	8.7	1.8	5.8	17	1.5	5.5	0.5	0.5	
75	163	138	7.3	20.0	11.2	9.1	1.9	8.1	17	1.5	5.5	0.5	0.5	
56	128	100	6.5	18.8	9.2	6.9	1.6	4.8	17	1.5	5.5	0.5	0.5	
61	149	123	8.0	20.1	10.4	7.2	1.6	5.8	17	1.5	5.5	0.5	0.5	
62	153	122	7.6	19.5	9.5	6.8	1.6	5.7	17	1.5	5.5	0.5	0.5	
77	162	138	6.5	19.8	10.1	6.2	2.0	7.5	17	1.5	5.5	0.5	0.5	
67	162	130	6.6	19.6	9.4	8.2	1.9	6.4	17	1.5	5.5	0.5	0.5	
56	147	119	7.1	19.6	9.9	6.6	1.7	5.4	17	1.5	5.5	0.5	0.5	
52	136	107	7.8	19.7	9.7	7.4	1.6	4.8	17	1.5	5.5	0.5	0.5	
75	150	125	6.8	20.7	11.0	7.3	1.7	7.5	17	1.5	5.5	0.5	0.5	147

TABLE XII

Experiment XII

	URINE								FAECES					BLOOD
quant ml/hr	total N mg/hr	urea mg N/hr	amm. mg N/hr	creatinine mg N/hr	S mg/hr	P mg/hr	K m eq/hr	Na m eq/hr	N mg/hr	S mg/hr	P mg/hr	K m eq/hr	N m eq/hr	urea mg/L
90	355	334	11.4	28.0	21.4	21.5	2.4	10.0	18	2.0	15.3	0.15	0.05	
96	435	394	12.1	[illegible]	25.4	19.5	2.8	8.0	18	2.0	15.3	0.15	0.05	244
97	420	379	11.9	[illegible]	[illegible]	25.6	2.1	5.8	18	2.0	15.3	0.15	0.05	
56	[illegible]	371	12.5	29.0	25.0	19.2	1.6	6.0	18	2.0	15.3	0.15	0.05	
64	423	380	13.4	25.8	23.9	20.6	2.3	5.9	18	2.0	15.3	0.15	0.05	
66	412	375	11.4	[illegible]	22.6	22.9	2.1	7.3	18	2.0	15.3	0.15	0.05	
[illegible]	421	384	13.6	[illegible]	[illegible]	19.6	2.3	5.6	18	2.0	15.3	0.15	0.05	
69	422	391	12.2	[illegible]	23.0	[illegible]	2.2	6.3	18	2.0	15.3	0.15	0.05	
48	[illegible]	375	12.2	27.9	22.9	18.1	1.9	5.5	18	[illegible]	15.3	0.15	0.05	
[illegible]	[illegible]	395	12.4	31.6	23.5	15.7	2.3	8.2	11	1.2	11.8	0.1	0.03	
33	[illegible]	267	17.3	[illegible]	20.6	11.8	1.0	3.0	11	1.2	11.8	0.1	0.03	
[illegible]	350	270	[illegible]	28.0	19.9	9.5	1.3	4.2	11	[illegible]	11.8	0.1	0.03	
33	[illegible]	310	16.3	28.0	[illegible]	12.8	[illegible]	5.6	11	1.2	11.8	0.1	0.03	
60	377	325	[illegible]	29.2	[illegible]	14.3	1.3	7.9	11	1.2	11.8	0.1	0.03	238
64	379	325	13.4	28.4	19.9	14.1	1.7	7.5	11	1.2	11.8	0.1	0.03	
79	[illegible]	370	11.8	[illegible]	21.8	19.3	2.1	8.8	11	[illegible]	11.8	0.1	0.03	233
70	[illegible]	364	11	[illegible]	22.9	[illegible]	2.3	7.0	11	1.3	11.8	0.1	0.03	
[illegible]	[illegible]	400	[illegible]	[illegible]	22.5	[illegible]	2.3	7.7	11	[illegible]	11.8	0.1	0.03	
98	430	379	14.3	28.3	22.8	22.7	1.9	6.8	11	1.2	11.8	0.1	0.03	
66	445	384	12.7	[illegible]	[illegible]	20.9	[illegible]	6.7	11	1.2	11.8	0.1	0.03	48

TABLE XIII

EXPERIMENT XIII

	FOOD						MED	
date 1955	*calories /day*	*N mg./kg*	*S mg./kg*	*P mg./kg*	*K m.eq./k*	*Na m.eq./kg*		*body weight kg*
March								
25	2450	98	4.8	8.3	2.0	6.5		65.7
26								65.5
27								65.2
28								65.0
29								64.8
30								64.4
31								64.0
April								
1								64.45
2								64.1
3							TP 250 mg + Hyaluronidase 100	64.0
4								63.8
5								64.0
6								64.[illegible]
7								64.3
8								64.25
9								61.9
10							Hyaluronidase 100 u.	61.6
11								61.4
12								61.2
13								61.2

TABLE XIII

Experiment XIII

	URINE									FAECES					BLOOD
quant. ml /hr	total N mg /hr	urea mg N /hr	ammon. mg N /hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m eq. /hr	Na m eq /hr	N mg /hr	S mg /hr	P mg /hr	K m eq. /hr	Na m eq. /hr	urea mg./L
72	244	198	11.1	26.5	17.4	17.0	3.0	6.9	26	1.9	8.0	0.3	0.1	
95	262	208	10.1	27.0	17.4	14.7	2.8	7.2	26	1.9	8.0	0.3	0.1	174
64	239	184	14.4	26.5	17.1	16.0	2.6	5.9	26	1.9	8.0	0.3	0.1	
68	253	196	13.4	26.1	17.0	16.2	2.5	6.2	26	1.9	8.0	0.3	0.1	
78	245	195	9.3	26.5	16.8	15.4	2.5	7.2	26	1.9	8.0	0.3	0.1	
64	239	191	12.6	26.5	16.3	16.0	2.5	6.3	26	1.9	8.0	0.3	0.1	
46	222	172	11.4	25.5	16.2	12.5	1.9	5.1	26	1.9	8.0	0.3	0.1	
67	230	185	15.9	26.5	16.5	14.2	2.2	6.9	26	1.9	8.0	0.3	0.1	
53	222	174	12.8	26.9	14.9	12.8	1.9	5.9	26	1.9	8.0	0.3	0.1	
57	238	178	12.4	28.2	16.8	11.4	2.1	7.1	22	1.8	6.3	0.2	0.1	
33	192	137	13.4	25.5	14.9	12.1	1.5	4.6	22	1.8	6.3	0.2	0.1	
37	178	129	10.5	25.3	13.9	8.1	1.3	5.5	22	1.8	6.3	0.2	0.1	143
42	164	117	11.9	24.6	13.3	3.9	1.2	4.7	22	1.8	6.3	0.2	0.1	
42	190	145	11.4	24.7	13.2	4.3	2.0	7.2	22	1.8	6.3	0.2	0.1	
67	210	168	10.8	25.3	12.4	6.4	2.0	7.1	22	1.8	6.3	0.2	0.1	156
82	222	184	9.9	24.9	13.1	10.0	2.6	7.8	22	1.8	6.3	0.2	0.1	
80	218	182	13.0	24.9	12.9	12.0	2.6	6.5	22	1.8	6.3	0.2	0.1	
74	218	176	10.0	24.7	11.9	15.0	2.3	6.7	22	1.8	6.3	0.2	0.1	
62	218	170	10.4	24.6	13.1	13.1	2.3	6.4	22	1.8	6.3	0.2	0.1	
77	215	179	10.3	25.2	12.6	12.0	2.5	7.0	22	1.8	6.3	0.2	0.1	165

TABLE XIV

EXPERIMENT XIV

	FOOD							MED	
date 1955	*calories /day*	*N mg./kg*	*S mg./kg*	*P mg./kg*	*K m.eq./kg*	*Na m.eq./kg*	*Cl m.eq./kg*		*body weight kg*
Oct									
25	2450	98	4.8	8.3	2.0	6.5	0.8		69.9
26						6.5			69.5
27						6.5			69.2
28						6.5			69.05
29						6.5			69.0
30						6.5			68.9
31						6.5			68.9
Nov									
1						6.5			69.0
2						6.5			69.0
3						6.5		NAPP 125 mg.	68.8
4						6.5			68.9
5						6.5			68.8
6						6.5			68.8
7						6.5			68.9
8						6.5			68.8
9						6.5			68.65
10						20.7			68.5
11						20.7			68.7
12						20.7			68.75
13						20.7			68.6
14						20.7			68.35
15						20.7			68.05
16						6.5			68.0
17						6.5			67.5
18						6.5			67.1

TABLE XIV

Experiment XIV

period of hr	total N mg /hr	urea mg N /hr	ammon. mg N /hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m.eq /hr	N m.eq /hr	Ca m.eq. /hr	N mg /hr	S mg /hr	P mg /hr	K m.eq /hr	Na m.eq /hr	C m.eq /hr	urea mg /L	Ca m.eq. /L
	URINE									FAECES						BLOOD	
[illegible]	234	204	7.9	22	15.2	14.3	1.5	6.6	0.6	16	1.4	3.6	0.1	0.1	0.03	388	4.65
[illegible]	216	176	6.7	22	17.1	12.0	1.7	5.7	0.58	16	1.4	3.6	0.1	0.1	0.03		
73	215	176	7.2	25	14.6	12.8	2.3	6.8	0.64	16	1.4	3.6	0.1	0.1	0.03		
79	218	177	6.7	25	16.7	16.4	2.5	6.5	0.65	16	1.4	3.6	0.1	0.1	0.03		
77	198	158	7.6	24	14.0	13.9	2.5	6.3	0.58	16	1.4	3.6	0.1	0.1	0.03		
73	184	146	7.2	23	13.6	13.3	2.3	5.5	0.57	16	1.4	3.6	0.1	0.1	0.03		
63	179	141	6.7	24	13.7	13.4	2.2	5.0	0.52	16	1.4	3.6	0.1	0.1	0.03		
[illegible]	173	137	8.4	24	13.8	13.7	2.5	5.4	0.61	16	1.4	3.6	0.1	0.1	0.03		
[illegible]	193	164	7.3	24	13.5	14.8	2.5	7.8	0.65	16	1.4	3.6	0.1	0.1	0.03		
80	167	133	12.3	24	12.8	10.0	1.8	5.4	0.53	23	1.9	4.4	0.1	0.2	0.03	139	4.98
67	171	131	6.6	25	16.3	10.6	2.0	6.4	0.48	23	1.9	4.4	0.1	0.2	0.03		
[illegible]	159	123	8.7	25	14.6	9.0	1.8	5.0	0.54	23	1.9	4.4	0.1	0.2	0.03		
[illegible]	149	112	7.9	24	14.8	11.1	1.9	5.5	0.43	23	1.8	4.4	0.1	0.2	0.03		
55	140	108	5.1	24	14.0	10.9	1.7	6.1	0.52	23	1.9	4.4	0.1	0.2	0.03		
[illegible]	147	108	6.9	24	15.7	13.4	1.9	6.1	0.58	23	1.9	4.4	0.1	0.2	0.03		
72	147	119	6.2	24	14.2	7.9	1.8	7.4	0.58	23	1.9	4.4	0.1	0.2	0.03		
63	143	112	5.8	23	13.4	8.7	2.1	7.7	0.62	9	0.8	2.5	0.1	0.1	0.02	114	4.90
61	154	105	7.3	24	14.0	13.5	3.0	13.8	0.73	9	0.8	2.5	0.1	0.1	0.02		
65	164	117	7.8	24	12.2	19.0	3.1	21.3	0.84	9	0.8	2.5	0.1	0.1	0.02		
67	157	113	9.2	4	12.4	15.2	2.6	20.7	0.84	9	0.8	2.5	0.1	0.1	0.02		
75	158	113	11.1	24	12.6	14.3	2.6	19.3	0.71	9	0.8	2.5	0.1	0.1	0.02		
79	157	111	10.0	24	11.8	13.5	2.4	18.9	0.78	9	0.8	2.5	0.1	0.1	0.02		
62	154	112	7.1	24	11.8	8.2	1.5	10.6	0.66	12	1.2	2.9	0.1	0.1	0.02	115	4.70
73	159	116	7.6	23	11.7	10.7	1.6	7.2	0.57	12	1.2	2.9	0.1	0.1	0.02		
65	184	142	6.0	22	12.6	12.8	2.1	6.3	0.52	12	1.2	2.9	0.1	0.1	0.02	136	4.95

TABLE XV

date	headache	palpitations	oedema	itching	vomiting	blood pressure	albuminuria	haemoglobin g	blood protein	blood urea mg/L	urea clearance	calories /day	NaCl	proteins	blood transfusion
1947						160 90				352	50		low		
1949						160 90							low		
1951						160 90							low		
1953						160 90							low		
June 1955						180/105		10.8		1620	10 0		low	low normal bread	1 L.
Jan 1956						185/110		10 4	normal	1865	5 4	2500	very low	18 g /day	1.5 L.
March 1956		–	–			140 90		14.1		863	4.5	2500	very low	18 g /day	
July 1956				–		160/95	+	10.1	normal	904		2500	very low	18 g./day	1 L.
Oct. 1956	–		–	–	–					980		2500	very low	18 g./day	
Nov 1956	+		–	–	–			10.1		1485	4 0	2500	very low	15 g./day	2 L.
Jan. 1957	–	–	–		–	180/120	+	14.9		1041	3.5	2500	very low	15 g /day	
Febr 1957	+	–	–	–	–	180/120	+	13.8		1090		2500	very low	15 g./day	
March 8 1957	+ +	+	+	+	+ +	210/130	+			2075			very low		
March 10 1957	+ +	+ +	+	+ +	+ +	230/135	+			3730			very low		
March 24 1957	†														

TABLE XVI

Experiment XV

date 1956	FOOD: calories /day	FOOD: N mg /hr	MED + date	URINE: body-weight kg	URINE: quant ml/hr	URINE: total N mg /hr	URINE: urea mg N /hr	URINE: creatinine mg N /hr	URINE: S mg /hr	URINE: P mg /hr	URINE: K m.eq /hr	URINE: Na m eq. /hr	URINE: Ca m eq. /hr
Febr 9–11	2540	120		71 0	66	149	111	1[illegible]9	12.1	10.[illegible]	1 44	1.06	0.11
12 14				70.0	56	134	95	12.4	11 7	8.6	1.36	0.88	0 09
15–17			Febr 15 NAPP 125 mg.	70.0	58	135	99	13.9	14 1	8.5	1.35	0.76	0.10
18–20				70.1	56	126	90	13.4	13.0	8.6	1 18	0.69	0.09
21 23				70.2	56	114	81	12.4	13 6	7.5	1.22	0.85	0 09
24–26				70 5	61	110	78	11 7	11.2	7 1	1 12	1 0[illegible]	0 10
27–29				70.3	58	108	74	12.0	13.1	6.5	1.24	0 87	0 08
March 1 3				70 0	54	103	69	11 6	11.0	6 2	1.2[illegible]	0 70	0 10
4–6				69.5	53	99	66	11.8	12.4	6.5	1.29	0.87	0 06
7–9				69.5	54	94	65	11.5	10.9	6.5	1.24	0.95	0 08
10–12			March 10 NAPP 125 mg.	69.3	49	84	60	11.2	9.2	6.1	1 18	0.73	0 09
13–15				69.5	48	83	57	11.[illegible]	9.9	6.0	1 15	0.41	0.14
16–18				70.1	63	95	63	12.3	11.0	6.5	1.25	0.83	0.12
19–21				69.2	61	98	61	11 0	10.3	5.5	1.28	0.93	0.12
22–24				69 1	56	90	56	10.8	10 4	5.8	1 18	1 01	0.11

TABLE XVI

Experiment XV

FAECES						BLOOD						
N mg/hr	S mg/hr	P mg/hr	K m eq/hr	Na m eq/hr	Ca m eq/hr	urea mg/L + date	protein total g/L	album. g/L	pseudo-globul. g/L	euglob. g/L	haem. g %	urea clear. γ
59	4.0	20.2	0.16	0.04	2.66	Febr 9 1400	69.3	40.2	17.8	11.3	10.4	4.6
59	4.0	20.2	0.16	0.04	2.66							
62	6.1	22.2	0.17	0.07	3.20	Febr 15 1367					11.4	4.6
62	6.1	22.2	0.17	0.07	3.20							
62	6.1	22.2	0.17	0.07	3.20	Febr 21 1129						4.2
62	6.1	22.2	0.17	0.07	3.20	Febr, 24 1109						
62	6.1	22.2	0.17	0.07	3.20	Febr 27 1173						
62	6.1	22.2	0.17	0,07	3.20	March 1 1128						
62	6.1	22.2	0.17	0.07	3.20	March 4 1115						4.2
62	6.1	22.2	0.17	0.07	3.20	March 7 1033					11.0	
62	5.5	20.1	0.19	0.07	3.18	March 10 1000						
62	5.5	20.1	0.19	0.07	3.18	March 13 883						4.5
6[illegible]	5.5	20.1	0.19	0.07	3.18	March 16 851						
62	5.5	20.1	0.19	0.07	3.18	March 19 889						
62	5.5	20.1	0.19	0.07	3.18	March 24 917	70.4	40.4	15.9	14.1	11.5	3.9

TABLE XVI

EXPERIMENT XV

date 1956	FOOD: calories /day	FOOD: N mg /hr	MED: + date	URINE: body weight kg	quant ml./hr	total N mg /hr	urea mg.N hr	creatinine mg N /hr	S mg /hr	P mg /hr	K m eq /h	Na m eq /hr	C m eq. /hr
Febr 9–11	2540	120		71 0	66	149	111	12.9	12.1	10.2	1 44	1 06	0 11
1 –14				70.0	56	134	95	12.4	11 7	8.6	1.36	0.88	0.09
15–17			Febr 15 NAPP 125 mg.	70 0	58	135	99	13.9	14 1	8.5	1.35	0 76	0.10
18–20				70.1	56	126	90	13 4	13.0	8.6	1 18	0.69	0.09
21 23				70 2	56	114	81	12.4	13 6	7.5	1.22	0 85	0.09
24–26				70.5	61	110	78	11 7	11.2	7 1	1 12	1.02	0.10
27–29				70.3	58	108	74	12.0	13.1	6.5	1.24	0.87	0.08
March 1 3				70 0	54	103	69	11 6	11.0	6.2	1.22	0 70	0 10
4–6				69.5	53	99	66	11.8	12.4	6.5	1.29	0.87	0 06
7 9				69.5	54	94	65	11.5	10.9	6 5	1.24	0.95	0 08
10–12			March 10 NAPP 125 mg.	69.3	49	84	60	11.2	9.2	6.1	1 18	0.73	0.09
13–15				69.5	48	83	57	11.2	9 9	6.0	1 15	0 41	0.14
16–18				70.1	63	95	63	12.3	11.0	6.5	1.25	0.83	0 12
19–21				69.2	61	98	61	11 0	10.3	5.5	1.28	0.93	0 12
22 24				69 1	56	90	56	10.8	10.4	5.8	1 18	1.01	0 11

TABLE XVII

Comment XVI

FAECES				BLOOD					
V mg/hr	S mg/hr	P mg/hr	K m eq/hr	urea mg./L. + date	protein total g/L	album. g/L	pseudo-glob. g/L	euglob g/L	urea clear %
51	5.9	18.9	0 2	Nov 29 1460	70 3	42.8	12.3	15 2	4.3
51	5 9	18.9	0.2						
51	5 9	18 9	0.2						
51	5 9	18.9	0.2	Dec. 9 1340					4.3
51	5 9	18 9	0 2	Dec. 12 1283					
51	5 9	18 9	0.2						
48	5 7	19 7	0.2	Dec. 17 1240					3 8
48	5 7	19 7	0.2	Dec. 20 1180					
48	5.7	19 7	0.2	Dec. 23 1205					
48	5.7	19.7	0.2	Dec. 26 1201					
48	5.7	19 7	0 2	Dec. 31 1166					3 6

TABLE XVII

EXPERIMENT XVI

day 1936	FOOD calories /day	FOOD N mg /hr	MED + day	body-weight kg	URINE quant ml./hr	URINE total N mg /hr	URINE urea mg.N /hr	URINE creatinine mg.N /hr	URINE S mg /hr	URINE P mg /hr	URINE K m.eq /hr	URINE Na m.eq /hr	URINE Cl m eq. /hr
Nov.–Dec.													
29–1	2540	100		64.5	52	177	95	8.8	9.9	7.3	1.24	1.40	1.50
2–4				65.0	52	120	95	8.5	10.1	6.7	1.30	1.09	1.30
5–7				64.5	60	136	101	9.1	11.4	7.7	1.45	1.69	1.80
8–10				63.8	54	123	93	9.3	10.9	7.0	1.53	1.47	1.69
11–13				63.6	45	102	78	7.8	9.3	5.5	1.[illegible]5	1.12	1.24
14–16				63.4	44	93	67	8.2	9.1	5.5	1.09	1.22	1.15
17–19			Dec. 17 NAPP 125 mg.	63.2	44	91	65	8.4	8.7	6.0	1.08	1.06	1.07
20–22				63.3	47	85	60	8.1	8.9	6.1	0.98	1.33	1.36
23–25				63.7	53	85	60	8.4	9.6	6.2	1.00	1.58	1.55
26–28				63.3	50	85	61	8.4	9.3	6.2	1.02	1.42	1.42
29–31				63.0	49	86	63	8.4	9.6	6.0	0.98	1.40	1.35

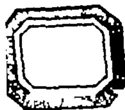
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STUDIES ON HUMAN SERUM β-LIPOPROTEINS, INCLUDING THEIR PROTEIN MOIETY

By

KIM CRAMÉR



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GÖTEBORG 1961

CONTENTS

To my wife

DR. GUN CRAMÉR, M. D.

I gratefully dedicate this work in recognition
of her aid and helpful criticism at a time when
her own work has been burdensome.

The present thesis is based on the following papers

I. Cholesterol and Phospholipid Contents of Human β-Lipoprotein in Different Lipemic States and Following Myocardial Infarction.
J Atheroscler. Res. *1* 317 1961

II Characterization of β-Lipoprotein Isolated from Hydroxylapatite Columns.
(together with L. Brattsten)
J Atheroscler Res *1* 335 1961

III Serum β-Lipoprotein Lipids and Protein in Normal Subjects of Different Sex and Age. With a Note on the Separation of β-Lipoproteins by Chromatography on Hydroxylapatite.
Acta Med. Scand. In the press.

IV Serum β-Lipoprotein Lipids and Protein During Combined Administration of Dioxydiethylstilbestrol and Methyl Testosterone.
Acta Med. Scand. in the press.

V *Serum β-Lipoprotein* Lipids and Protein During Administration of Triparanol.
Acta Med. Scand. in the press.

VI Changes in Serum and Lipoprotein Fatty Acids During Administration of Ethyl Arachidonate.
(together with P Björntorp)
Acta Med. Scand. In the press.

In the following these publications are referred to under their Roman numerals.

INTRODUCTION

The association between the lipids from serum or plasma and protein was first suggested by Nerking in 1901 (1), but it was not until 1929 that Macheboeuf (2) isolated a more or less purified protein fraction containing lipid by means of acid precipitation of horse plasma. Blix, Tiselius, and Svensson (3) demonstrated that a similar preparation was divided into two parts in free electrophoresis — one migrating with the α-globulins and the other with the β-globulins. Pedersen's discovery (4) that a protein containing lipid could be brought to the surface of the tube in preparative ultracentrifuge led to the isolation methods introduced by Gofman et al (5) and to the employment of the analytical ultracentrifuge for estimation of serum lipoproteins. Meanwhile, cold-ethanol precipitation methods were introduced by Cohn et al (6) which yielded preparations containing two different classes of lipoproteins. Separation by means of zone electrophoresis on paper and on starch was introduced by Swahn (7) and by Kunkel (8) respectively.

Precipitation methods with different sulfated polysaccharides were introduced by Burstein (9), Oncley et al (10), and Bernfeld et al (11) while Carlson (12) used chromatographic procedure. Qualitative and quantitative estimates of β-lipoproteins by means of precipitation with β-lipoprotein antisera has also been made (13).

A great number of publications employing the above-mentioned methods and several modifications thereof have appeared. It has been established that two main fractions of lipoproteins can be isolated. One has a density of more than 1.063 and migrates with the α-globulins during all kinds of electrophoretic procedures. It is called "high density lipoprotein" (HDL) or "α-lipoprotein". The other fraction has a density of less than 1.063 and can be subfractionated both electrophoretically and by means of ultracentrifugation. The main portion of the fraction migrates with the β-globulins while the minor portion may migrate with the α_2-globulins, or as a "pre β" fraction.

Several density classes can be defined in the preparative ultracentrifuge. "Svedberg flotation classes" = Sf classes are defined in the analytical ultracentrifuge. A summary of these subdivisions and the relationships between the different methods is given in Table I.

The borderline is not distinct between the lipoproteins with the lowest density, i.e. about 1.00, and the "chylomicra" which appear in serum of hyperglyceridemic patients and in normal persons when they have consumed a meal containing fat. There is mounting evidence (14) that one part of the chylomicra in some way is associated with the α-lipoproteins, while the other part is related to the β-lipoproteins described above. These matters are discussed more closely in Paper III.

Table I
Summary of different isolation procedures for β-lipoproteins and their interrelationships

Preparative ultracentrifuge density class	Analytical ultracentrifuge Sf class	Cohn fractionation	Electrophoresis			β-lipoprotein antiserum precipitation	Dextrane sulfate precipitation
			free	paper	starch		
0.96 – 1.006	20–400	I+III	β_1 or α_2	β_1 *)	α_2(?)	+	partly (?)
1,006–1.019	12– 20	I+III	β_1	β_1	β_1	+	+
1,019–1.063	0– 12	I+III	β_1	β_1	β_1	+	+

The table has been prepared based on the review of Cornwell and Kruger (31).
*) according to Perold et al. (37)

The lipoproteins consist of a lipid moiety containing cholesterol, phospholipids, and glycerides, and a protein moiety. The protein moiety is antigenically similar in all lipoproteins with a density of less than 1.063 (15, 16) except in the alimentary chylomicra (17).

The analytical ultracentrifuge gives no information as to the composition of the lipoproteins. Pure fractions suitable for chemical analyses, may be obtained from the preparative ultracentrifuge if sufficiently long runs are made (18, 19, 20) or if some concentrating measure is applied to the preparation such as COHN fractionation (21) or precipitation with dextrane sulfate (10). It is difficult, however, to employ these methods for the study of larger groups, because of the complicated procedures necessary for preparation. This is true especially if it is the intention to determine the protein moiety of the lipoprotein as well, as it is necessary to prolong the ultracentrifugation over several days to avoid contamination by other proteins. The reports of such determinations are also comparatively few. A list is found in Table VII of Paper III.

The interest in the β-lipoproteins is caused primarily by their probable importance in the development of atherosclerosis (22). There is, however, no proof that any special component of the lipoproteins should be responsible for this effect. Elevated levels of β-lipoprotein cholesterol as well as elevations of the serum glycerides (23) or of the lipoproteins with a density of less than 1.019 (24) have been found in coronary heart disease. There is no evidence that these elevations are primary or secondary to other metabolic disorders. Probably neither cholesterol, nor glycerides may be kept in solution in serum without a protein moiety. It was therefore of interest to study the concentration of this moiety in various states, as well as the interrelationships between the different components of the β-lipoproteins.

PRESENT STUDY

In this paper all lipoproteins with a density of less than 1.063 will be called β-lipoproteins, except the alimentary chylomicra. This is partly based on practical considerations and has been supported by authoritative workers in the same field (22, 25, 26).

The aim of this study was to determine whether changes in the composition of the β-lipoproteins occur with age, whether differences exist between the sexes, and whether rapid changes of the β-lipoprotein level lead to a change in the proportions between the components of the β-lipoproteins. The distribution of polyunsaturated fatty acids in the lipoproteins was also studied, as was the effect of administration of a tetraenoic acid, arachidonic acid.

It was considered of special interest to attempt to determine the concentration of the protein moiety as no studies on such concentration changes have been published so far.

I. Isolation Methods

Plans for the present work were outlined in 1957 after a study of essential hypercholesterolemia by WELIN AND CRAMÉR (27) and owing to the necessity of getting a better definition of the nature of the serum cholesterol and its relationships to the other components of the lipoproteins. Studies in our laboratory (28) based on COHN fractionation had confirmed that the bulk of the serum cholesterol was found in the fraction I + II + III, i.e. the β-lipoproteins. This technique did not permit determinations of the protein moiety because of contaminations. Precipitation methods with sulfated amylopectin (11) and dextrane sulfate (10) did not yield quantitative results with those preparations of the precipitating agents which were available. The precipitation method with heparin and phenol (29), published in 1958, however, appeared to give a quantitative recovery of β-lipoprotein lipids also from hypercholesterolemic sera (Paper I).

In 1959 HJERTÉN (30) published a description of the isolation of β-lipoproteins by means of chromatography on hydroxylapatite. All serum proteins, except the β-lipoproteins, could be eluted with a 0.25 M phosphate buffer with pH of 6.8, and the β-lipoproteins were then eluted with a 0.65 M phosphate buffer with the same pH. This method had a high capacity and seemed well suited for the isolation of sufficient amounts of β-lipoproteins for reliable analyses, also when determinations of protein nitrogen were made by means of the micro-KJELDAHL method.

a. The Chromatographic Method

In Paper II it was demonstrated that the chromatography of serum on hydroxylapatite yielded a preparation of immunolo-

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1.019—1.063	0— 12	I+III	β_1	β_1	β_1	+	+

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Table II

Comparison between cholesterol and phospholipid contents of β-lipoproteins isolated by means of heparin-phenol precipitation and by chromatography on hydroxylapatite in normal subjects.

		Cholesterol mg/100 ml		Phospholipids mg/100 ml		cholesterol / phospholipid molar ratio	
		Prec.	Chrom.	Prec.	Chrom.	Prec.	Chrom.
Normal males	20—40 years $n = 14$	127 ± 9	123 ± 9	91 ± 6	103 ± 7	2.78 ± 0.05	2.39 ± 0.06
	p					< 0.001	
	55—65 years $n = 11$	134 ± 8	173 ± 10	101 ± 6	125 ± 7	2.68 ± 0.05	2.80 ± 0.07
	p	< 0.001		< 0.001			
Normal females	20—40 years $n = 15$	105 ± 5	101 ± 5	79 ± 4	88 ± 4	2.68 ± 0.03	2.32 ± 0.07
	p					< 0.001	
	55—65 years $n = 11$	168 ± 6	187 ± 11	121 ± 5	139 ± 9	2.80 ± 0.04	2.71 ± 0.05

Prec. = heparin-phenol precipitation
Chrom. = hydroxylapatite chromatography
The differences were tested according to the t-test and the p values are indicated where significant differences were found.

On the other hand, SCANU *et al.* (29) in the description of the applied method, found identical values for cholesterol and phospholipids in the precipitates and in the top layer after ultracentrifugation of the same serum at a density of 1.063. BURSTEIN AND SAMAILLE (35) also considered the precipitation with heparin quantitative, if manganese chloride was used for precipitation.

In order to test the completeness of the precipitation as a function of lipoprotein density the ratio between chromatographed and precipitated phospholipids was correlated to the level of glyceride found in the chromatographed preparation of the same serum. The phospholipid rather than cholesterol were chosen, as the lipoproteins with a density of less than 1.019 contain comparatively more phospholipids than cholesterol. A significant correlation was found in the older normal material ($n = 22$, $r = 0.57$, $p < 0.01$). Thus, precipitated phospholipids decrease in amount with increasing β-lipoprotein glyceride, i. e. conditions which SCANU *et al.* described for hyperlipemic sera are to some extent valid also for normal sera. The age of the normal persons in the material of SCANU *et al.* was not defined.

This finding supports the completeness of the chromatographic method of separation.

The β-C/P ratios, given in Paper I, are probably valid only for that part of the β-lipoprotein pool which has a density of 1.063 — 1.019. The latter figure may how-

gically homogeneous β-lipoproteins with negligible protein contaminations. The chromatography separated lipoproteins with densities between 1 063 and < 1 006.

A study of Borgström *et al* (31) on rat chyle lipoproteins has shown that lipoproteins of the Sf class 400—1000 are eluted with a 0 7 M phosphate buffer while those of Sf > 1000 are trapped in the columns. In the present study it could be demonstrated that no additional lipid was eluted with a 1 M buffer once elution with a 0 65 M buffer had been completed.

Ingestion of fat did not influence the level of the chromatographed lipoprotein during the postprandial phase (Paper III). This is in conformity with the data of Havel and Fredrickson (32) which were based on experiments with dogs who were given labeled palmitate, and also to some extent with the study of Borgström *et al* (31) on rat chyle. Half of the specific activity which they found in the Sf 20—1000 class was trapped in the columns, while the other half was eluted with a 0 7 M buffer

Recently Bergquist *et al* (33) utilizing a β-lipoprotein antiserum for isolation (13) made an observation on postprandial sera similar to that of the present study A correlation between that method and the present one, which yields an immunologically pure product, thus appears to exist.

In Paper III the proportions between serum and hydroxylapatite were also adjusted, and the adequate amount of 0.25 M buffer was determined in order to obtain a quantitative recovery of β-lipoproteins from serum. As no pure β-lipoprotein preparation was available, the recovery was considered quantitative when no precipitation lines against β-lipoprotein antiserum were obtained from the 0.25 M eluates, and when no material containing lipid or protein could be found after addition of more 0 65 M buffer or 1 M buffer The amounts of cholesterol and phospholipids, which could be extracted from the columns after the chromatography were negligible.

b Comparison Between the Applied Methods for β-Lipoprotein Isolation

The older normal materials described in Paper I and III were identical, while the younger materials were not. Analyses of precipitated β-lipoproteins, however, were carried out on the younger material described in Paper III parallel with the analyses of the chromatographed lipoproteins.

A comparison between the results is shown in Table II (one young male is not included).

The β-lipoprotein cholesterol/phospholipid ratios (β-C/P ratios) which were found were lower in the chromatographed preparations from the younger materials than in the precipitated ones, while no difference existed between the older groups. The isolated amounts of cholesterol and phospholipid were lower after precipitation than after chromatography in the older groups. This held true also for the phospholipids in the younger groups.

Cornwell and Kruger (34) have reviewed the precipitation methods with sulfated polysaccharides, and have concluded that most methods precipitate the β-lipoproteins of the Sf 0—9 class, while the lipoproteins of higher Sf classes are not precipitated completely

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This finding supports the completeness of the chromatographic method of separation.

The β-C/P ratios, given in Paper I, are probaly valid only for that part of the β-lipoprotein pool which has a density of 1.063 – 1.019 The latter figure may how-

ever, be somewhat lower than 1 019. Different states of disease may influence the β-lipoproteins in several ways and render them non-precipitable.

This appears to be true especially in the case of acute myocardial infarction. In five patients, parallel analyses with both methods were carried out during the acute stage of an infarction. In 14 specimens from the first week, the phospholipid values were, on an average, 31% higher in the chromatographed preparations than in the precipitated ones. There was, however, no correlation between the β-lipoprotein glycerides and the ratio between chromatographed and precipitated phospholipids. A certain correlation was found between the β-C/P ratios from both methods ($r = 0.57$, $p < 0.05$). As was pointed out in Paper I, the β-C/P ratios found after precipitation during the first two days, could be explained by the disappearance of all lipoproteins of Sf classes above 12 (density less than 1 019). Evidently these lipoproteins are still found in serum but not precipitated.

The lack of correlation between the degree of precipitation and the glyceride level makes it probable that a defective precipitation may be caused also by other factors than the glyceride content of the β-lipoproteins. Such factors are then apparently active as early as a few hours after a myocardial infarction, and may precede the infarction.

The β-C/P ratios found during administration of estrogen and androgen hormones, as described in Papers I and IV, were found to be decreased both when determined after precipitation and when determined after chromatography. Larger variations between pure estrogen therapy and estrogen+androgen were found after precipitation than after chromatography. The materials, however, were not identical.

II. Results of the Chromatographic Separations of the Lipoproteins

a. β-Lipoprotein Composition

A comparison between the observed composition of the β-lipoproteins and data from previous investigations is given in Paper III. If the average weight percentage composition of the present preparations is calculated with the assumption that 25% of the cholesterol is unesterified, and if the molecular weights of 387 for cholesterol, 669 for cholesteryl esters, 775 for phospholipids and the factor 14×6.25 for the calculation of peptide weight from protein nitrogen re used, the following figures were found to represent the mean of the normal materials in paper III

	cholesterol free	cholesterol ester	phospholipid	glyceride	peptide
of total weight	7 5	38.5	22	9	23

These values agree with those based on ultracentrifugal separations, which were cited in Paper III, and are presented here for a more convenient comparison with these studies in which the composition as rule was given in terms of weight.

Ultracentrifugal preparations of pure fractions, however, are time-consuming and expensive. The advantage of the chromatographic method of isolation is that it is comparatively simple and that it yields a product which does not need further purification. These properties have made possible the study of larger materials and the drawing of conclusions as to changes within the β-lipoproteins with age or during experimental conditions.

The changes with age, described in Paper III, showed an increased ratio between cholesterol and protein in both sexes, while the ratio between phospholipids and protein remained unchanged. The cholesterol/protein ratio was somewhat higher in older males than in older females, and the glyceride/protein ratio was lower in young females than in young males. A close relationship between protein and phospholipid was also indicated by the figures in Paper IV which showed that the most evident changes during the administration of methyl testosterone were seen in these two components. The phospholipid/protein ratios in the patients in Paper IV were also within the limits of the normal material, and were independent of treatment with different hormones, while their cholesterol/protein ratios varied widely Nor did the change in the sterol component, induced by triparanol as described in the study of Paper V influence the phospholipid/protein ratio.

b. The Polyunsaturated Fatty Acids of the Lipoproteins.

In Paper VI, the fatty acids in serum and in the cholesteryl esters, the phospholipids, and the glycerides + the free fatty acids have been determined before and after the administration of arachidonic acid in two survivors of myocardial infarction. The largest percentage increases in this acid (determined as tetraenes) were found in the phospholipids of both the α and β-lipoproteins. It is known that the phospholipids

interchange freely between these lipoproteins (36) Similar changes were also found in the cholesteryl ester fatty acids of α and β-lipoproteins, while the glycerides showed an increase of the arachidonic acid percentage only in the β-lipoproteins. The α lipoprotein glycerides were virtually unaffected by the dietary changes.

A decrease of percentage dienes was seen concomitantly with the increase of arachidonic acid The main dienoic acid is linoleic acid, which acts as a precursor to arachidonic acid. The decrease of this acid after exogeneous supply of arachidonic acid could indicate that this is one of its main functions.

c β-Lipoprotein Structure

It appears as if the phospholipids and the protein are closely related to one another in the β-lipoprotein molecules, and that cholesterol or other sterol components are present in varying amounts. This may be caused by a "reserve" capacity of the lipoproteins for the transport of cholesterol, or to changes within the protein component induced by age or by therapeutic measures.

The correlation between cholesterol and protein, however when calculated on homogeneous groups as in Paper III was not weaker than the correlation between phospholipids and protein. This may point to a structural relationship to the protein for cholesterol as well as for the phospholipids within the molecules. A further identification of this relationship can not be made until more details on the β-lipoprotein structure are available.

The association of the glycerides with the β-lipoprotein molecules, however, is not as clear in normal individuals. The correlation found between glycerides and other β-lipoprotein components in older females is an isolated finding, and should not be over evaluated until more evidence of a difference between this group and the others is available.

Table III

Molar relationships between β-lipoprotein lipid components and estimated amount of amino acid residues in the β-lipoprotein

	Age group Number of subjects	Molar ratio lipid/10 amino acid residues		
		cholesterol	lipid phosphorus	glycerides
Normal males	20—40 years n=15	3.25	1.36	0.54
	55—65 years n=11	4.16	1 43	0 49
Normal females	20—40 years n=15	3 16	1.36	0.39
	55 65 years n=11	3 91	1.39	0.51

It is at present impossible to state whether the regulation of the protein moiety or the regulation of any of the lipid components is the most decisive factor in determining the level of the β-lipoproteins in serum.

The amino acid composition of the lipoprotein protein has been defined by SHORE AND SHORE (37). Calculating on the basis of their figures, which are valid for the Sf 6 class, 10 moles of protein nitrogen would be equivalent to about 8 moles of amino acid residues. This means that the molar lipid/protein ratios in Papers III—V should be multiplied by 1.25 to give the approximate molar ratio between lipid and 10 amino acid residues. The figures for the normal materials are shown in Table III.

The figures in Table III would mean an approximate molar relationship cholesterol. phospholipid:glyceride:amino acid residue of e.g. 32.14.4.100 in young females and of 40.14.5.100 in older persons of both sexes. As the question of the sterical configuration of the lipoproteins is far from solved, the above figures can at present be used as a basis for speculations only.

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My association with Dr. Bertil Hood M.D and Dr. Gunnar Welin M.D forms the background to this work. It was carried out at Medical Service I at Sahlgrenska Sjukhuset, Göteborg, where I have the privilege of being a member of a group under the stimulating leadership of Dr. Hood.

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SUPPLEMENTUM 371

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K. L. BECKMANS BOKTRYCKERI

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Subject Groups Number of Subjects and Time of Examination

Group	Number of Subjects	Time of Examination
A. Conscripts		
1 At registration	320	Oct—Nov 1937
	170	1938
	70	1950
	73	1950
2. At induction		
) Selected on registration	27	May 1938 (registered 1937)
	47	1950 (1957)
	14	1050 (1958)
Total	88	
(b) Ra domly chosen on in duction	178	May 1960 (registered 1958—50)
3 Duri g military service		
(a) Selected on registration	27	Aug 1958—Mar 1959 (registered 1957)
	47	Aug 1959 (1057)
	14	1950 (1958)
Total	88	
(b) Randomly chosen during milit ry service	201	Dec 1050 (registered 1857— 58)
B Naval cadets		
1 At the aptitud tests	63	April—May 1950
2 After three month training	63	Aug 10 0
C. GIS trainees		
1 At start of course	48	Oct 1038—Oct 1939
— After one month training	48	Nov 1938—N v 1059
3. After four months training	41	Mar 1959—Mar 1960
D Weight lifters, wrestlers and runners		
1 Weight lifters nd wrestlers	48	Dec 1958—Nov 1959
2. Runners	28	May—Nov 1959
T tal of examinations	1 533	

Abbreviations

I 1 — Kungliga Svea Livgarde (the Svea Lifeguards — an infantry regiment).

KSS — Kungliga Sjökrigsskolan (Royal Swedish Naval College)

GIS — *Försvarets gymnastik och idrottsskola* (Swedish Armed Forces Physical Training School).

PWC — Physical working capacity (kpm/min at heart rate 1 0 beats/min).

Relative PWC — Physical working capacity per kilogram of body weight.

Registration — *Enrolment for military service.*

Induction — Call up for military service

Total Hb — Total amount of hemoglobin in the body

INTRODUCTION

The classification of conscripts in connection with registration for military service is founded partly upon a fairly comprehensive psychologic examination which provides a rough idea of the intelligence level, and partly upon a general medical examination the results of which serve as a basis for division of the men into groups. However assignment of various military duties might be greatly facilitated by additional means of classification based on physical capacity—in which respect the routine medical examination currently affords no guidance Data relating to physical capacity—i.e., information concerning *the muscular strength of the arms and legs, the circulatory* and respiratory capacity as well as the endurance or stamina—may well be of significance in the assignment of military duty It is necessary to consider not only the particulars recorded at the time of registration but also the registrant s developmental potential (results of body growth, effects of various types of physical activity etc.)

Investigations of national servicemen with regard to physical working capacity and the effects of physical activity have been conducted by various uthors (Wahlund 1948 Lindegård, 1956 Linroth 1957 and others) Alth ugh these nd other studies have yielded very comprehensive inf rmation, there remains a number of problems which appear to be of great practical and theoretical significance For example grading of the phy ical capacity in large groups of conscripts requires the use f methods which are both accurate enough and imple enough to be practicable A further search for uch method is necessary Another problem complex pertains t the exercise capacity and the muscular strength in average series of young conscript on registration or on call-up f r national service There i the question of whether these factors currently differ from those bserved in previous years and of whether they are closely correlated to ge A third major question is whether the values for the physical w king capacity can be predicted with sufficient accuracy from certain reulat ry parameters (e.g the heart volume) or from physical characteri tics (g height, skeletal type, body weight, etc) A fourth problem con plex concerns the responses to physical exercise of series of

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Chapter I

METHODS OF EXAMINATION

A. General Procedure

The examinations were carried out over a period dating from the autumn of 1957 to the spring of 1960 Weight lifters and wrestlers were examined largely in the evenings other subjects in the mornings and afternoons

Following annotation of the name, address and age a blood sample was taken from the finger tip for determination of the hemoglobin concentration. After a brief interval auscultatory examination of the heart, followed by recording of the blood pressure was undertaken with the subject prone.

The anthropologic measurements comprising height, femoral condylar breadth, body weight, muscular strength, and thickness of subcutaneous fat (skinfold thickness) were thereafter performed

In each instance the heart volume was estimated prior to the work test. Determination of the total hemoglobin was also done, as a rule before the test, though in some cases it was deferred until one of the following days. Lastly the physical working capacity was recorded.

In a series of 43 conscripts chosen at random duplicate determination were carried out with all methods at intervals of 1—2 hours The standard deviation of each difference as well as the coefficient of variation was annotated

B Data Concerning Age, Place of Residence, Occupation, Physical Activity and State of Health

1 The *age* of each subject was recorded as the number of full years from the year of birth to the year of examination, months being disregarded.

2. In annotating the *place of residence*, Stockholm (the place of examination) was taken as reference point. On the basis the residence was recorded as (a) the City and County of Stockholm (called, in the following, the Stockholm area) (b) provincial towns, and (c) rural districts.

3. For the purposes of this investigation the *occupation* was the type of work by which the examinee had earned his living. For conscripts in military service the last civilian occupation was recorded. When the

subjects which differ from each other in physique and in the degree to which they are physically trained—the training here referring both to that which develops muscular strength and to that which chiefly develops the circulatory capacity As regards the effects of physical training another question which arises is whether the responeses of athletes whose choice of sports is presumably founded in some measure on specific bodily and/or mental traits, differ from those of average individuals It would be of particular interest to know whether the effects of physical training are markedly correlated to the skeletal dimensions and whether measurement of the latter might enable one to predict the degree of increase in physical capacity that may result from a given type of physical training

This dissertation is concerned with the above-mentioned problem complexes Large series of conscripts were examined in different years, then followed up from the ages of 18 to 19 or in some cases, 20 years. In addition minor groups were followed up during the course of military service in order to observe the effects of the physical activity described in the following The responses to another type of physical activity were studied in a series of naval cadets Also investigated was a third main group comprising selected officers and warrant officers from the Swedish Armed Forces Physical Training and Sports School (GIS) whose responses to very hard physical exercise were studied. Lastly two groups of athletes were compared in respect to physical capacity body build and circulatory capacity One of these groups consisted of runners who could be expected to have a particularly high circulatory capacity the other consisted of weight lifters and wrestlers in whom exceptionally high muscular strength was to be expected

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and 4 7 mm Hg (coefficient of varia tion 4.3 per cent)

D Anthropologic Determinations

Lindegård (1953, 1956) showed that the body build can be approximately defined on the basis of the skeletal build, the amount of muscular tissue and the amount of fat tissue. The modes of measurement employed in his investigations were, in part, modifications of previous methods (Martin, 1928 Stolz & Stolz, 1951) The present anthropologic studies followed the main lines reported by the above-mentioned authors, though minor modifications were introduced All determinations were car ried out by the writer The measur ing instruments were calibrated at the Division of Electrical Machinery Royal Institute of Technology Stock holm They are illustrated in figure 1 A—E.

1 *The skelet l build* can be approxi mately characterized by a length factor and a sturdiness factor of the skeleton (Lindegård 1953, 1956) The sturdiness is determined by the magnitude of appositional bone growth nd the length by endochon dral bone growth Characteri tics of th skeletal build can thus be objectively recorded by mea urement f these factors.

In this investigation the apposi tional bon growth wa determined I y mea uring the width of the right femoral condyle The width was re gi tered a the linear distance be tween the medial and lateral condylar surfaces, with the callipers (see figure 1 E) pressed lightly against the condyle. For this procedure the subject was seated in a chair with his knee bent to an angle of 90 degrees and his lower leg vertical, the entire foot resting on the floor The measurement was taken perpendicularly to the long axis of the leg. The maxi mum breadth was recorded in millimetre steps. The standard deviation of the difference between two conse cutive determinations was found to be 0 06 cm (coefficient of variation 0 4 per cent) $(n = 43)$

For measurement of the height the subjects were barefooted and the stature was recorded to the nearest 0.5 cm. Since the body height does not constitute an exact measure of the skeletal length, including as it does the factors of appositional bone growth, thickness of articular car tilage and curve of the spine, its use as a criterion of the skeletal length is not entirely correct. The body height, however is not only well cor related to the skeletal length but in cludes a larger component of the length factor than of the stur diness factor in practice theref re it can be used for the grouping of subjects. Since moreover the body h ight is readily determinable with complete objectivity it was here chosen to represent the length factor The standard deviation of the difference between two consecutive deter minations was found to be 0.54 cm (coefficient of variation 0.2 per cent) $(n = 43)$

examiner found it difficult to judge in what measure the vocation had been physically demanding the subject himself estimated the degree of physical effort involved in his work The results of this subjective evaluation were taken as a basis It was thus stated whether the work was to be regarded as (a) heavy manual work (b) moderately heavy manual work, (c) light manual work, or (d) sedentary work

4 *The examinees were required to* state whether they had pursued any form of *physical exercise* The latter was graded as (a) competitive sport (b) active sport and (c) no sport. Those who had engaged in competitive or active sports were then asked if they had done so during the preceding six months only those who answered in the affirmative were assigned to the »athletic» group Since it was difficult to judge whether the demands of the particular forms of sport affected primarily the muscular strength of the physical working capacity no differentiation was undertaken in this respect

5 All examinees were questioned as to their *state of health* They were asked for example, if they had any history of syncope attacks of tachycardia pains in the chest on effort collapse as a result of exertion It was also ascertained whether they had suffered any acute febrile disease in the preceding two weeks and whether they considered themselves healthy at the time of examination.

C Physical Examination

1 Careful auscultatory examination of the *heart was undertaken in all* subjects The findings were classified as (a) negative (b) suspectedly pathologic, and (c) pathologic

2 The *blood pressure* was likewise *determined by the auscultatory* method in all subjects at each examination. Due to lack of time the examinees were unable to rest prior to the blood pressure reading which was invariably taken from the left arm via a mercury manometer and with the subjects supine on an examining couch The diastolic pressure coincided with the point at which the Korotkow sounds abruptly lost intensity The values were recorded in 5 mm Hg steps

Blood pressures were taken by various examiners, all of them physicians In one series of 50 subjects, two physicians recorded the blood pressure independently one about half an hour after the other and each making duplicate determinations For one of them the standard deviation of the difference between two consecutive determinations was 3.5 mm Hg systolic (coefficient of variation 2 0 per cent) and 4 0 mm Hg diastolic (coefficient of variation 3 8 per cent) for the other the corresponding figures were 4.5 mm Hg (coefficient of variation 2.5 per cent)

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For measurement of the height the subjects were barefooted and the stature was recorded to the nearest 0.5 cm. Since the body height does not constitute an exact measure of the skeletal length, including as it does the factors of appositional bone growth, thickness of articular cartilage and curve of the spine, its use as a criterion of the skeletal length is not entirely correct. The body height, however is not only well correlated to the skeletal length but includes a larger component of the length factor than of the sturdiness factor In practice, therefore, it can be used for the grouping of subjects. Since, moreover the body h ight is readily determinable with complete objectivity it was here chosen to represent the length factor The standard deviation of the difference between two consecutive determinations was found to be 0.54 cm (coefficient of variation 0.2 per cent) ($n = 43$)

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4 The examinees were required to state whether they had pursued any form of *physical exercise* The latter was graded as (a) competitive sport, (b) active sport and (c) no sport. Those who had engaged in competitive or active sports were then asked if they had done so during the preceding six months only those who answered in the affirmative were assigned to the »athletic» group Since it was difficult to judge whether the demands of the particular forms of sport affected primarily the muscular strength of the physical working capacity no differentiation was undertaken in this respect

5 All examinees were questioned as to their *state of health* They were asked for example if they had any history of syncope attacks of tachycardia pains in the chest on effort collapse as a result of exertion It was also ascertained whether they had suffered any *acute febrile disease* in the preceding two weeks and whether they considered themselves healthy at the time of examination

C. Physical Examination

1 Careful auscultatory examination of the *heart* was undertaken in all subjects. The findings were classified as (a) negative, (b) suspectedly pathologic, and (c) pathologic

2 The *blood pressure* was likewise determined by the auscultatory method in all subjects at each examination Due to lack of time the examinees were unable to rest prior to the blood pressure reading, which was invariably taken from the left arm via a mercury manometer and with the subjects supine on an examining couch. The diastolic pressure coincided with the point at which the Korotkow sounds abruptly lost intensity The values were recorded in 5 mm Hg steps.

Blood pressures were taken by various examiners all of them physicians In one series of 50 subjects, two physicians recorded the blood pressure independently one about half an hour after the other and each making duplicate determinations For one of them the standard deviation of the difference between two consecutive determinations was 3.5 mm Hg systolic (coefficient of variation 2 0 per cent) and 4 0 mm Hg diastolic (coefficient of variation 3 8 per cent) for the other the corresponding figures were 4.5 mm Hg (coefficient of variation 2.5 per cent)

Both the skeletal sturdiness and the body height may show major variations, a circumstance which has led to the use of the terms heavy skeletoned and light skeletoned This classification is frequently subjective and does not take sufficient account of the relationship of sturdiness to height. By determining however the ratio of body height to skeletal sturdiness—termed, in the following, the skeletal quotients—this relationship is taken into account. Persons with identical skeletal quotients thus have equally heavy skeletons in relation to height, though the two factors may have different absolute values.—In this investigation values ranging from approximately 16 to just over 20 for the skeletal quotient were obtained. The standard deviation of the difference between two consecutive determinations was 0 14 (coefficient of variation 0.6 per cent) ($n = 48$)

In some instances the skeletal build was taken as a basis for the grouping of individuals. Those with skeletal quotients exceeding the mean thus constituted one group, and those with quotient falling short of the mean another Each of these groups was then subdivided with respect to height. From this procedure emerged four subgroups with different skeletal characteristics i.e., (a) tall heavy-skeletoned, (b) tall, light-skeletoned, (c) short, heavy-skeletoned, and (d) short, light-skeletoned. This basis of classification was chosen because the skeleton is subject, during adulthood, to only slight alteration as compared to that associated with other parameters, e.g. muscular development and thickness of subcutaneous fat.

2 *Muscular strength* was determined on the general lines reported by other authors (Stolz & Stolz, 1951 Lindegård, 1953, 1956) The right and left hand grip as well as the shoulder pull and shoulder thrust were measured. Three determinations were made for each muscle group, the highest value being taken to represent the muscular strength. The measuring equipment employed is illustrated in figure 1 A B and C.

It is highly probable that the amount of muscle tissue in any given part of the normal body is proportional to the total muscle tissue Exeptions of course exist in persons who have highly developed muscle groups involving a relatively greater volume of muscle tissue It seems justifiable, however to assume as a working hypothesis that the amount of muscle tissue, and hence the muscle strength, in one part of the body such as an extremity is proportional to the total muscle tissue and, accordingly to the total muscular strength (Lindegård 1953) This in any case should be valid for groups of subjects chosen at random

For all determinations of strength Collins dynamometer (see figure 1 A—C) was, as in previous investigations (Stolz & Stolz, 1951 Lindegård, 1953, 1956) employed. The dynamometers used here were, however fitted with specially de-

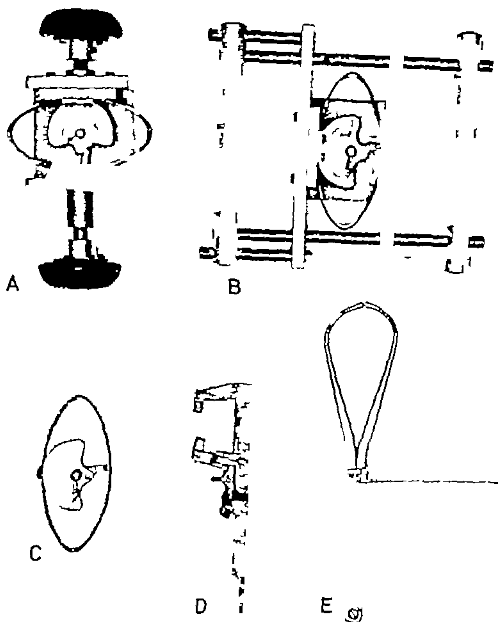


Figure 1

A Apparatu for d t rmination of muscular strength (shoulder thrust)
B Apparatu f r d terminati n of muscular strength (shoulder pull)
C Apparatus for d termination of muscular strength (ha d grip)
D Apparatus fo determinati n f skinf ld thickness
E. Apparatus f determinati n f fem ral condylar breadth

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signed cogwheel mechanisms (AB Instrumenta Lund) The muscular strength was recorded in dynamometer units which were then converted into *kiloponds* (Tekniska Nomenklaturcentralen Stockholm) During the course of the investigation the dynamometers were calibrated five times at regular intervals. The initial calibration was done during the autumn of 1958, and it was subsequently possible to record values for muscular strength In no case did the values obtained in dynamometer units directly accord with the values in *kiloponds*

On the basis of the five calibrations a mean curve was plotted for each of the dynamometers. All values for the muscular strength were recorded in *kiloponds* taking this mean curve as a basis The maximum deviations from the mean curve to the individual calibration curves were ± 5 per cent for shoulder thrust ± 2 per cent for shoulder pull and ± 2 per cent for the right and left hand grip within the relevant measurement ranges By using a mean curve of the five calibrations as well as specially made cogwheel mechanisms in the dynamometers, the apparatus error was kept to a minimum

The standard deviation of the difference between two consecutive determinations was found to be 3.3 kp (coefficient of variation 5.2 per cent) for the right hand grip 3 8 kp (coefficient of variation 0 8 per cent) for the left hand grip 3 9 kp (coefficient of variation 6 2 per cent) for shoulder pull and 2 4 kp (coefficient of variation 2.3 per cent) for shoulder thrust ($n = 43$)

3 The thickness of the subcutaneous fat *(skinfold thickness)* was measured by a previously devised method (Keys & Brozek, 1953 Skerlj Brozek & Hunt Jr., 1953) also used by Lindegård (1956) Skinfolds were measured at three different points on the right side of the body namely (a) on the back just caudal to the scapula (region I) (b) at the axillary border of the pectoralis major muscle at the level of the mammilla (region II) and (c) on the abdomen in the midclavicular line at umbilical level (region III) In each instance, three determinations were made and the mean was taken as the skinfold thickness in the pertinent region The values were recorded in millimeters with an accuracy of 1 mm. The measuring appliance employed is illustrated in figure 1 D

It has been shown (Lindegård 1956) that a positive significant correlation exists between truncal and extremital skinfold thicknesses Determinations on the trunk as described above thus provide indirect information concerning the extremities too It has also been demonstrated (Lindegård 1956) that no significant correlation exists between skinfold thicknesses on the upper and lower parts of the trunk Since, however in the present investigation measurements were made on both parts of the trunk the re-

corded values represent the fat distribution in the trunk *per se*

The apparatus used for measuring skinfold thickness was also calibrated On recording of the values the spring had a torsional moment of 161 gcm. The surface area of each caliper tip applied to the skin was 16 sq mm. By applying a lever at the pivot and loading it with a known weigth at a constant distance from the pivot, a continuous check was secured to ensure that the spring tension did n t change during the course of the investigation. No adjustment was necessary The standard deviation of the difference between two consecutive determinations was found to be 1 00 mm (coefficient of variation 7.9 pe cent) for region I, 0.93 mm (coefficient of variation 9.3 per cent) for region II and 0.50 mm (coefficient of variation 9 1 per cent) for region III ($n = 43$)

4 The *body weight* was taken with the subject wearing shorts and was ecorded to the nearest 0 1 kg below th indicated weight. Calibration of the scales showed that the indicated weights were consistently 0 035 kg too high No correction was made fo this error of apparatus The tandard deviation of th difference between two body weight determinations separated by an interval not e ceeding two hours was 0 10 kg (coefficient of ariation 0 1 per cent) ($n = 43$)

E Laboratory Procedures

1 The *physical working capacity* (PWC) was determined by methods devised at Karolinska Sjukhuset (Sjöstrand, 1947 1950 1951 1960 Wahlund, 1948 Holmgren *et al* 1957) Each examination comprised recording of heart rate and electrocardiogram at rest prior to the work test an orthost tic test, a work test, and recording of heart rate and electrocardiogram after the work test.

Prior to the work test, heart rate and electrocardiograms were taken after ten minutes rest, leads I, II, III, CR_1, CR_2, CR_4, CR_5, and CR_7 being used for the latter examinations

In the ortohostatic test the heart rate and electrocardiograms were recorded after the subject had been standing against a wall for eight mi nutes with his feet and the back of his head in contact with the wall

For estimation of PWC the subject performed a work test on an elect rically braked bicycle ergometer designed by Holmgren & Mattsson (1954) The test opened with a load of 300 kpm/min, the load subsequently being increased by 300 kpm/min at si -minute intervals. Heart rate and lectrocardiograms were taken after 2, 4 and 6 minutes, nd the respiration rate after three minutes with each load. The load was increased until a heart rate of 170 beats per minute in relative teady stat (defined as a change of ten beats or less in the heart rate from the second to the sixth minute

of the work load) The PWC value was *obtained by graphic intra and* extrapolation, using the linear relationship between heart rate and exercise load *In* some instances the work test was discontinued *before* the subject had reached a heart rate of 170 since from the values ob*tained it was found reasonable to* estimate the working capacity at 170/min by extrapolation. In these cases the final heart rate at the highest load invariably *exceeded 150 and* was usually over 160 beats per minute. In a number of subjects [9 9 *per cent of the conscripts at the time* of registration (1958 $n = 170$) 5 0 per cent at the time of induction *(1960* $n = 178$*)* and 6 8 per cent during military service (1959 $n =$ 204)] steady state was not reached at the highest load In such cases *the absolute PWC value found* on extrapolation to a heart rate of 160 beats per minute was regarded as *the physical working capacity*

Experience gained at Karolinska Sjukhuset suggests that a PWC value of 900 kpm/min is an approximate lower limit for healthy adults males (Wahlund, 1948 and others) The City of Stockholm Health Survey of 1954 (Frisk *et al* 1957) moreover yielded results which indicate that a PWC of 1 050 kpm/min is an approximate average *for healthy adult* males between the ages of 40 and 50 in a large urban population

Immediately after the work *test* as well as four minutes later the heart rate and electrocardiograms were taken in the same way *as* before The heart rate was again determined ten minutes after completion of the work *test*

All electrocardiograms were taken by a direct recording apparatus *(Mingograf 42 Elema Järnh, Stockholm)* The heart rate was determined from electrocardiograms for which the calibrated paper speed was 25 mm per second The interval covered by ten beats was measured by means of a rule which directly *converted the value to beats per* minute This method was used for all heart rate determinations except *the one made ten minutes after* the work test the value here being estimated by 30-second palpation of *the radial artery*

Respiration rates were determined by auscultation over the trachea for a period of 30 seconds, and recorded in *respirations per minute*

Four bicycle ergometers were used in the investigations. They were ca*librated twice annually the first* calibration being done prior to the investigation Table 1 shows the average *and percentual variations of* the calibrations for the four ergometers In no case did the variation exceed 3 1 per cent

2 *Total hemoglobin* was determined by the alveolar CO method of *Sjostrand* (1948) with the modifications reported by Carlsten *et al* (1954) Wiklander (1956) and Linderholm *et al* (1956 1957) Duplicate determinations separated by an interval of not more than one week were the *rule The subjects* examined in this

way were thletes, three of whom reported that they smoked sporadi cally In two subjects three deter minations were required because of leakage. — For adult males a mean of 11—12 g/kg body weight is regarded as an normal value (Holmgren *et al.*, 1957) —The standard deviation of the difference between two consecutive determinations was 16.5 g (coefficient of variation 3.9 per cent) ($n = 55$) In duplicate deter mination the standard error will thus be 2.8 per cent of the mean. This figure is consistent with those found in earlier in estigations (Sjöstrand 1948 Hallberg, 1955 Wik lande 1958)

3 The *hemoglobin concentration* (g/100 ml) was determined in blood samples taken from the finger tip, duplicate determinations being made in all instances. One quarter of a milliliter of blood was hemolyzed in 5 ml 0.04 per cent ammonium solu tion The readings were taken by spectrophotometry at 540 mμ and with the se of van Slyke s analyses of the O_2 and CO capacity as stan dard The tandard deviation of the difference between two consecutive d terminations was 0.36 g/100 ml (coeffici nt of variation 2.9 per cent) ($n = 50$) In duplicate determination the standard erro will thus be 2 1 per cent f the mean.

4 The *blood volume* was calculated on the ba is f the total hemoglobin nd hemoglobin concentration va lues. The hemoglobin concentration was assumed to be uniform throughout the vascular system It was not taken into account that due to regional disparities the body hematocrit probably constitutes not more than approximately 90 per cent of the hematocrit in the great blood vessels and of that in blood obtained by finger prick —With this method a mean of 82 ml/kg body weight has been found in adult males (Holm gren *et al.* 1958) —The standard deviation of the difference between two consecutive determinations was 0 40 1 (coefficient of variation 4.5 per cent) ($n = 55$)

5 The *heart volume* was determined with the subjects prone by the method of Larsson & Kjellberg (1948) as modified by Kjellberg *et al* (1949 1951) Exposures were thus made in two planes separately No pecial consideration was paid to the contraction phase of the heart as it has been shown (Kjellberg *et al* 1951) that the influence of the car diac cycle on the heart volume is inappreciable in the prone posi tion. Further exposures were made while the subjects took small shallow breaths ad modum Larsson & Kjellberg (1948) The stan dard deviation of the difference between two consecutive determinations was 44 ml (coefficient of va riation 3.9 per cent) ($n = 43$) This accords with earlier calculations (Kjellberg *et al* 1951) in which the error of method was found to be approximately 4 per cent.

F Statistical Methods

Current statistical methods were employed for calculation of the arithmetic mean the standard deviation the standard error of the mean for t tests, and for studies of correlations determined by regression analyses (Kemp 1955) Random selection, where undertaken was based on a statistically verified random digits table (Wallis & Roberts, 1956)

Statistical Symbols

n = number of observations
M = arithmetic mean
SD = standard deviation
e_M = standard error of the mean
r = correlation coefficient
V_M = coefficient of variation (SD in per cent of M)
e_{xy} = standard error of estimate
$diff$ = difference between two means
P = probability

Levels of Significance

x 0 05 > P > 0 01	Probably significant
xx 0 01 > P > 0 001	Significant
xxx 0 001 > P	Highly significant

Chapter II

SUBJECT GROUPS

All subjects used in this investigation had passed medical examinations, on the basis of which pathologic cases had been discarded. The selectivity which thus resulted accounts for the subsequent paucity of pathologic findings only two pathologic electrocardiographic reactions to the work test were noted among the conscripts examined on registration and induction, and one was recorded on examination of conscripts during military service. These cases too were discarded, the investigation being concerned with normal healthy persons. Two other subjects were excluded because of pper respirat ry tract infection associated with slightly elevated temperature.

Data obtained regarding previous illnesses and symptoms—e.g., histories of syncope, attacks of tachycardia, chest pains, obscure dyspnea, or collapse in connection with effort—in no case disqualified a subject for examination.

A. Conscripts on Registration and Induction, and During Military Service

1 *Registration.*—Conscripts 18 years f age were examined in connection with registration for military service. A total of 320 were seen in the autumn of 1957 170 in the autumn of 1958, 70 in the autumn of 1959 and 73 in the autumn of 1960 All were resident in the Stockholm area and were taken from a single conscription zone the registry of which deals with approximately 3000 conscripts annually Since under the existing law a conscript cannot be rdered to undergo examinations other than those normally connected with registration, all men who took part in the present investigation were volunteers. In order to expedite the start of each day's examinations, reference was given to men who got through the registration procedures most rapidly i.e., those with the best health records. Lastly only groups 1 and 2 were included. Hence, the conscripts annually examined out of the aforementioned 3000 were amongst those with the best health records, and had volunteered to undergo tests of the physical working capacity

2. *Induction*—In the spring of 1960 approximately 900 national servicemen were inducted into the Svea Lifeguards in Stockholm (an infantry regiment abbreviated in the

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Levels of Significance

^{x}0 05 > P > 0 01	Probably significant
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B Naval Cadets at the Royal Swedish Naval College (KSS)

Of the 88 naval cadets enrolled at KSS in the spring of 1959 63 were examined both in connection with their aptitude tests and on completion of three months schooling and physical training Thirty-eight per cent of them hailed from the Stockholm area 51 per cent from provincial towns and 11 per cent from rural districts.

C. Trainees at the Swedish Armed Forces Physical Training School (GIS)

From amongst the applicants—primarily officers and warrant officers —for the annual course at GIS a number are selected on the basis of experience as physical training instructors and on athletic aptitude. The course comprises both theoretical instruction and practical exercises, and terminates with a three-weeks period of training in the mountains. Each course has a total duration of approximately four months The men taking these courses constitute a selection of athletically inclined military personnel.

Two different year-groups were studied from the start to the finish of course. The first group consisted of 17 officers who began the course in the autumn of 1958 the second comprised 31 warrant officers who began it in the autumn of 1959 Since comparison of these two categories showed not only that they were similarly constituted with regard to body build but also that their muscular strength as well as their PWC values were approximately equal at the start and at the end of the course it seemed justifiable to consider them as a unit. The series thus consisted of 48 men who had undergone intensive physical training. They were examined at the start of the course, after one month and finally after four months—immediately following their return from three weeks training in the mountains. Each of the 48 men was examined on the first two occasions, but only 44 were available for the third examination, four being either sick or on leave Since, however the non inclusion of these four was in no way selective, it cannot have influenced the results to any appreciable degree.

Twenty nine per cent of the 48 men who took part in these courses hailed from the Stockholm area 65 per cent from provincial towns and 6 per cent from rural districts. The men belonged to various regiments stationed in different parts of the country

D Weight Lifters Wrestlers, and Runners

Since the weight lifters and wrestler pursued sport that developed primarily their muscular strength these two categories were combined to form a group totalling 48 subjects. Eighty two per cent of them hailed

following I 1) On the basis of the random digits table 195 of them referable to groups 1 and 2 were taken for examination 98 were aged 19 and 97 aged 20 Ninety-seven of the former and 81 of the latter or a total of 178 were actually studied the remaining 17 (8 7 per cent) were unavailable because of deferment of military service transfer to other regiments, or sickness Since the exclusion of these 17 was thus in no way selective with respect to body build or physical working capacity it cannot have affected the overall results Of the 178 national servicemen 87 6 per cent were from the Stockholm area 5 6 per cent from provincial towns and 6.8 per cent from rural districts Since the majority came from the Stockholm area the results are largely representative for that and not for the rest of Sweden All men in this part of the investigation were examined within one week after call up for military service

3 *Military Service*—The 19- and 20-year-old conscripts who had been inducted into I 1 in the spring of 1959 were studied after six months military service i e., in December 1959 On the approximately 400 men referable to groups 1 and 2, a total of 220 were chosen at random in the same way as before Exactly one-half were 19 and the other half 20 years of age One hundred of the former and 104 of the latter were actually examined the remaining 16 (7.2 per cent) being unavailable ten (4.5 per cent) of them sick and six (2 7 per cent) away on leave Since only 7.2 per cent of the 220 were not studied and since they did not constitute a selection their exclusion could not have affected the results appreciably Of the total series 78 per cent hailed from the Stockholm area 8 per cent from provincial towns and 14 per cent from rural districts Because the majority were from the Stockholm area the results are principally representative for that region

4 *Minor Groups followed up during the Year of Military Service*—In addition to the above mentioned groups, a small number of conscripts were studied not only on registration and induction but during the course of military service Of those examined at the time of registration in 1957 twenty seven were tested again on induction into I 1 in the spring of 1958 They were subsequently examined on several occasions during the year of military service Forty seven of those studied in the autumn of 1957 were again examined on induction into I 1 in the spring of 1959 and then once more after three months military service Another group comprising 14 conscripts who had been examined on registration in 1958 were re-examined both on induction in 1959 and on the completion of three months military service A total of 88 conscripts were thus examined not only on registration and induction but also after three months military service

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Since the weight lifters and wrestlers pursued sport that developed primarily their muscular strength, these two categories were combined to form a group totalling 48 subjects. Eighty two per cent of them hailed

from the Stockholm area 12 per cent from provincial towns and 6 per cent from rural districts Thirty eight per cent were referable to the middleweight 29 per cent to the lightweight, and 33 per cent to the heavyweight categories The men had engaged in their respective athletic specialities for an average of six and a half years and had com peted in tournaments

The runners formed a group of 28 men who had competed over distances of 800 to 10 000 meters. Sixty-eight per cent of them were from the Stockholm area, 14 per cent from provincial towns, and 18 per cent from rural districts. All of them competed in recognized tour naments, and they had engaged in their athletic activities for an average of seven and a half years.

Chapter III

RESULTS

A. Conscripts

1. At the Registration Procedures of 1957–1960

In the years 1957 1958, 1959 and 1960 groups of respectively 320 170 70 and 73 eighteen-year-old conscripts were examined on registration for military service the examinations taking place in the months of October and November The total number of conscripts examined was thus six hundred and thirty three.

The results are set forth in table 2. The average PWC decreased progressively until 1960 when a somewhat higher value (929 kpm/min) was noted. The differences, however were in no case significant. As regards muscular strength expressed as shoulder thrust, the value of 61.9 kp obtained in 1958 and that of 61.0 kp in 1959 showed no significant difference Only in respect of height did the values for the respective series differ significantly the conscripts of 1958 being significantly taller than those of 1957 According to statistics published by the Central National Service Office a similar disparity was recorded for conscripts registered throughout the country The heights and weights reported in publications of 1957–1959 from the Central National Service Office are parenthesized in table 2, from which it may be seen that the conscripts in this investigation were, on the whole, both somewhat taller and somewhat heavier than the average for the entire country

The coefficient correlation between physical working capacity and heart volume was determined with all investigated conscripts combined in one group. This coefficient was found to be 0 41xxx with a standard error of estimate of 163 kpm/min. The regression line was satisfied by the equation $y = 0.696x + 369$

2. Development as from Registration until Induction and During Military Service

(a) *Conscripts Registered in the Autumn of 1957 and Called up in the Spring of 1958*

Just over 30 of the conscripts selected and examined on registration in 1957 were called up for military service with I 1 in May 1958 Twenty seven of them were examined both on registration and induction as well as several times during the course of military service.

Table 3 shows the results. As respects PWC, heart volume, resting

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that the average value for body weight became higher during both the interval between registration and induction and that between induction and completion of three months military service. These increases, however were not significant

(b) *Conscripts Registered in the Autumn of 1957 and Called up in the Spring of 1959*

Of the conscripts selected and examined on registration in the autumn of 1957 58 were inducted into I I in the pring of 1959 i.e., one year after those reported in section (a) Forty-seven of these 58 were examined not only on registration and induction but after three months military service as well.

Th results a set forth in table 4 Comparison with the group described in section (a) showed that the PWC values at corresponding times did not differ significantly The muscular strength, expressed as shoulder thrust, showed no significant increase as from the time of ind ction to a point three months thereafter The valu on induction w s 66.5 kp—significantly higher than that for the conscripts on registrat on in 1958 and 1959

Another finding in table 4 is that the height was greater on induction than it had been at registration, the difference being significant. It did not incre se significantly however during the first three months of mi litary serv ce — Th body weight also ose significantly in the interval between registration and induction, but showed no significant change during the first three months thereafter

(c) *Conscripts Registered in the Autumn of 1958 and Called up in the Spring of 1959*

Fourteen of the conscripts selected and examined at registration in the autumn of 1958 underwent re-examination on induction and once again three months later

The results of three sets of examinations are set out in table 5 As regards PWC, comparison with the corresponding values reported in sections (a) and (b) above shows no significant differences. The same is true of the muscular strength, expressed as shoulder thrust.

It is also seen from table 5 that both height and weight increased not only as from registration until induction, but during the first three months of military service The increases, however were not significant.

(d) *Features Common to the Conscripts Reported in Sections (a) – (c)*

Since the conscripts reported in the preceding three sections exhibited, on the whole, a similar development as from registration until induction, and also during the first three months of military service it seemed justifiable to treat them as a single series. The group thus formed 88 conscripts, all of whom had been selected and examined on registration, then re-examined on induction and after completion f three months military service

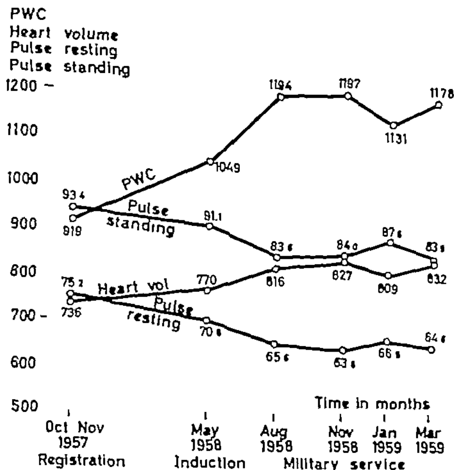


Figure 2
Conscripts examined at registration, inducti n and several times du ing military service

pulse and orthostatic pulse, the findings are also illustrated in figure 2 It is evident from both the table and the figure that the PWC rose during the interval between registration and induction, the increase being significant After three months military service moreover the PWC was significantly higher than on induction It subsequently remained more or less unchanged except for a non significant decrease during Christmas leave The heart volume, resting pulse and pulse in the erect position varied concurrently with the physical working capacity

It will be seen from table 3 that the muscular strength expressed as shoulder thrust, was 62 6 kp after six months military service. No significant change was found at the two subsequent examinations

Table 3 also shows that the height increased during the interval between registration and induction as well as between induction and the completion of three months military service For both periods the difference was significant. No significant increase in height was subsequently recorded.

From table 3 it is seen moreover

that the average value for body weight became higher during both the interval between registration and induction and that between induction and completion of three months military service. These increases, however were not significant.

(b) *Conscripts Registered in the Autumn of 1957 and Called up in the Spring of 1959*

Of the conscripts selected and examined on registration in the autumn of 1957 58 were inducted into I 1 in the spring of 1959 i.e., one year after those reported in section (a) Forty-seven of these 58 were examined not only on registration and induction but after three months military service as well.

The results are set forth in table 4 Comparison with the group described in section (a) showed that the PWC values at corresponding times did not differ significantly Th muscular strength expressed as shoulder thrust, showed no significant increase as from the time of induction to a point three months thereafter The value on induction was 66.5 kp—significantly higher than that for the conscripts on registration in 1958 and 1959

Another finding in table 4 is that th h ight was greater on induction than it had been t registration, the difference being significant. It did not ncrease significantly however during the first three months of military service.—The body weight also rose significantly in th interval between registration and induction, but showed no significant change during the first three months thereafter

(c) *Conscripts Registered in the Autumn of 1958 and Called up in the Spring of 1959*

Fourteen of the conscripts selected and examined at registration in the autumn of 1958 underwent re-examination on induction and once again three months later

The results of three sets of examinations are set out in table 5 As regards PWC, comparison with the corresponding values reported in sections (a) and (b) above shows no significant differences The same is true of the muscular strength, expressed as shoulder thrust.

It is also seen from table 5 that both height and weight increased not only as from registration until induction, but during the first three months of military service. The increases, however were not significant.

(d) *Features Common to the Conscripts Reported in Sections (a)—(c)*

Since the conscripts reported in the preceding three sections exhibited, on the whole, a similar development as f om registration until induc tion, and also during the first three months of military service it seemed justifiable to treat them as a single series. The group thus formed 88 conscripts, all of whom had been selected and examined on registration then re-examined on induction and after completion of three months military service

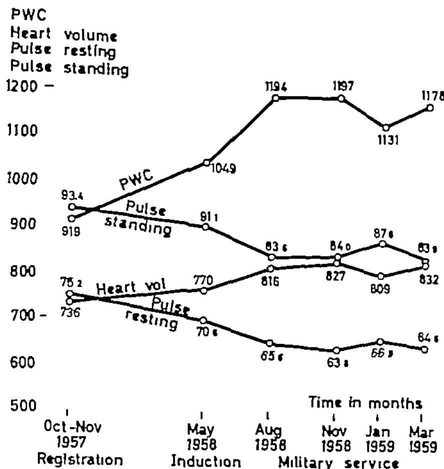


Figure 2

Conscripts examined at registr tion, induction and several times du ing military ervic

pulse and orthostatic pulse, the findings are also illustrated in figure 2. It is evident from both the table and the figure that the PWC rose during the interval between registration and induction, the increase being significant. After three months military service moreover the PWC was significantly higher than on induction. It subsequently remained more or less unchanged except for a non significant decrease during Christmas leave. The heart volume, resting pulse and pulse in the erect position varied concurrently with the physical working capacity.

It will be seen from table 3 that the muscular strength expressed as shoulder thrust, was 82.6 kp after six months military service. No significant change was found at the two subsequent examinations.

Table 3 also shows that the height increased during the interval between registration and induction as well as between induction and the completion of three months military service. For both periods the difference was significant. No significant increase in height was subsequently recorded.

From table 3 it is seen moreover

that the average value for body weight became higher during both the interval between registration and induction and that between induction and completion of three months military service. These increases, however were not significant.

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(d) *Features Common to the Conscripts Reported in Sections (a) —(c)*

Since the conscripts reported in the preceding three sections exhibited, on the whole, a similar development as from registration until induction and also during the first three months of military service it seemed justifiable to treat them as a single series. The group thus formed 88 conscripts, all of whom had been selected and xamined on registration, then re-examined on induction and after completion of three months military service

The findings for this combined group are detailed in table 6 In the interval between registration and induction height, weight PWC and PWC per kilogram of body weight (relative PWC) underwent significant increases These factors, with the exception of weight, also showed similar increases during the first three months of military service Moreover the resting pulse and the orthostatic pulse fell significantly during the interval between registration and induction as well as during the first three months of military service No significant differences were found on the other hand in regard to muscular strength and skinfold thickness.

The series was also studied with a view to ascertaining whether those conscripts who showed the greatest increase in PWC (1) between registration and induction and (2) during the first three months of military service, differed from the others in body build For this purpose the conscripts were divided into two groups, one comprising those whose PWC increased most, and the other those whose PWC increased least

At the time of induction 39 per cent of the conscripts who had been selected on registration showed an increase of PWC exceeding 100 kpm/min while 41 per cent exhibited an increase of less than 100 kpm/min These findings are presented in table 7 from which it will be seen that the conscripts whose PWC increased most were those with the lowest initial values In regard to height and weight on the other hand there were no significant differences between the two groups

As respects the increase in PWC during the first three months of military service (table 8) a rise exceeding 100 kpm/min occurred in 46 per cent and a rise of less than 100 kpm/min in 32 per cent. Here too, the greatest increases were found in those with the lowest initial values. The findings were similar for the relative PWC. In respect of height femoral condylar breadth and body weight on the other hand no significant differences emerged

The group of conscripts whose PWC increased least in the interval between registration and induction had a lower mean PWC than the others on induction The mean PWC found during military service was also in this group, lower than it had been on induction In neither instance, however was the difference significant

It will be seen from table 9 lastly that during military service the muscular strength expressed as shoulder thrust increased in 39 per cent but decreased in 38 per cent of the conscripts who had been selected and examined on registration. Those with increases had significantly lower values for muscular strength on induction With regard to height femoral condylar breadth weight and PWC no significant differences emerged between the two groups of conscripts

With a view to establishing

whether conscripts with a particular physique differed from the others in regard to the results, classification based on body build was undertaken. The skeletal type at the time of induction was taken as a basis. The ratio of height to femoral condylar breadth—the skeletal quotient—was in each case determined, following which the conscripts were classified in two groups—those with a skeletal quotient above and those with a quotient below the average value. Each of these two groups was then subdivided according to the mean height. This procedure yielded four subgroups with differing skeletal characteristics namely (1) tall, heavy skeletoned, (2) tall, light skeletoned, (3) short heavy-skeletoned and (4) short light-skeletoned conscripts.

The results for these four subgroups on registration on induction, and during military service are shown in table 10 which is implemented by figure 3 in respect of PWC, relative PWC, and muscular strength. Shoulder thrust is here considered representative of muscle strength, while the skinfold thickness is recorded only for region I. These factors were chosen because the methodologic error associated with their determination was, as pre-

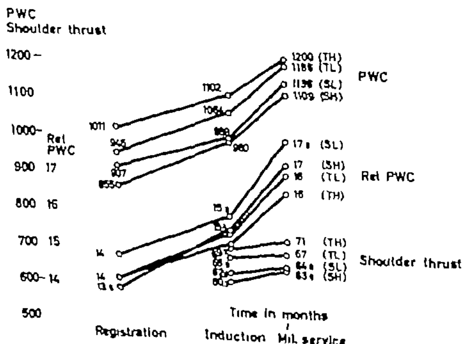


Figure 3
Conscripts divided into four subgroups based on skeletal build at induction
TH = Tall heavy-skeletoned TL = Tall light-skeletoned
SH = Short heavy-skeletoned; SL = Short light-skeletoned

viously shown less than that for the other parameters.

It is clear from table 10 and figure 3 that *tall heavy skeletoned* conscripts had on registration, higher PWC values than short, light-skeletoned and short heavy skeletoned conscripts The difference was significant in relation to the short heavy skeletoned subgroup and probably *significant to the short light* skeletoned subgroup The PWC on induction was also higher for tall heavy skeletoned conscripts than for the other three subgroups In respect of short conscripts the difference was probably significant Lastly the tall *heavy skeletoned subgroup showed* the highest PWC value even during military service the value however did not differ significantly from those for the other three subgroups

On registration on induction and during military service the short light *skeletoned subgroup had the* highest values for relative PWC. In no case, however did the values differ significantly from those for the other subgroup

It will be observed from figure 3 that tall conscripts had on induction the highest shoulder thrust values and that these increased during military service Short conscripts had lower values on induction but also showed an increase during military *service None of the differences*, however were significant

Those of the conscripts who on induction stated that they engaged in competitive sports or did heavy work were *examined separately in* order to ascertain if their body build differed from that of the others On induction 17 men (19 per cent) said they engaged in competitive sports In respect to height, femoral condylar breadth weight and PWC they did not differ significantly from the others During military service their PWC increased though not to a significant degree. Another ten conscripts (11 per cent) reported on induction, that they did heavy work The differences between these men and the others were not significant for height femoral condylar breadth, weight and PWC. During military service the *PWC increased though here too the* rise was not significant

The coefficients of correlation between PWC on the one hand and on the other heart volume, height femoral condylar breadth skeletal quotient and weight were determined The pertinent data referable to the time of registration, induction and three months after the start of military service are presented in table 11 The highest coefficient of correlation *was found between heart* volume and PWC on all three occasions The standard error of estimate amounted to 136 kpm/min on registration and 139 kpm/min on induction and during *military service*

Also determined were the corresponding coefficients between shoulder thrust on the one hand and on the other height, femoral condylar breadth *skeletal* quotient and weight The results are set forth in *table 12*. Both on induction and three

months thereafter the highest coef ficient of correlation was that be tween weight and shoulder thrust. The standard error of estimate was 9 7 kp on induction and 9 0 kp during military service

2. Two Additional Groups of Conscripts Examined on Induction and During Military Service

The collecting of a series of con scripts on registration could not be based upon a statiscally verified ran dom digits table It had to be assumed that the series constituted a select group with the best health re cords. With the aim of securing larger groups and making the results more representative, 178 additional onscripts were examined on induc tion int I 1 in the spring of 1960 as well as 204 thers on completion of six months military service with I 1 n the autumn of 1959 These two groups were collected on the basis of the random digits table and each contained approximat ly equal num bers f 19- and 20-year-old men.

No significant diff rences emerged between the two age groups, either n nduction r during military ser ice Hence they wer not treated separately

The find ngs recorded in the con scripts hosen t random on induc- ti n r presented n table 13, which also gi es the corresponding values for the conscripts selected at regist tion. The tw groups of subject xhibited on induction, no signifi cant difference ex ept in the case of houlder thru t, for which the randomly chosen conscripts had a lower value.

Table 14 shows the results observed in the conscripts chosen at random during military service, as compared with the previously reported series. The two groups exhi bited significant differences in skin fold thickness, the values for which were lower in the randomly collected group The latter moreover had a significantly highe systolic blood pressure Otherwise there were no statistically significant differences between the two series of conscripts on examination during military ser vice Comparison of the physical working capacity with that (1252 kpm/min) found by Linroth (1957) in 94 conscripts after eight months military service with I 1 in 1954 showed a significantly higher value for Linroth s group

The findings recorded for the randomly chosen groups on induc tion and during military service were also compared, the results being presented in table 15 The con scripts examined after six months military service had significantly higher values for PWC, relative PWC, heart volume, sh ulder thrust, and systolic blood pressure Their values were also higher though not significantly for shoulder pull as well as right and left hand grip Further they showed significantly lower alues for resting pulse, orthostatic pulse and skinfold thickness in region III. Lastl they had lower values for skinfold thickness in regions I and II and f r diast lic blood

pressure the differences here being probably significant. No other significant differences were noted between the two groups.

The series collected at random on induction and after six months military service were like the previously reported series classified according to skeletal build the four subgroups in each instance consisting of (1) tall heavy skeletoned (2) *tall light skeletoned* (3) short heavy skeletoned and (4) short light skeletoned conscripts

The values recorded in these four subgroups are presented in table 16 To clarify the differences between the series randomly collected on induction and after six months military service in regard to PWC, relative PWC, and muscular strength these factors are also illustrated in figure 4 The muscular strength is represented by shoulder thrust, and the skinfold thickness by the values for region I as with the conscripts selected on registration In figure 4 the differences for the four subgroups are distinguished by broken lines, *to signify that the two series* are referable to different contingents of conscripts.

It is evident from table 16 and figure 4 that differences existed between the four subgroups in each series of randomly chosen conscripts,

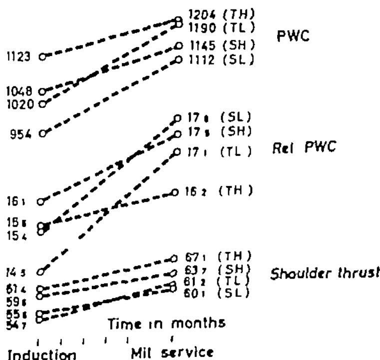


Figure 4

Conscripts divided into four subgroups based on skeletal build
TH = Tall heavy-skeletoned TL = Tall light-skeletoned
SH = Short heavy-skeletoned SL = Short light-skeletoned

i.e. those examined on induction and those examined after six months military service. Tall heavy-skeletoned men had on induction, a higher PWC than short light-skeletoned and tall light skeletoned conscripts, the difference being highly significant in the first instance and probably significant in the second. After six months military service tall heavy-skeletoned conscripts again showed a higher PWC than the other three subgroups, though the difference was significant only in relation to the short, light-skeletoned men.—At the time of induction the short, heavy skeletoned subgroup had the highest value for relative PWC. However the difference was significant only in relation to the tall, light skeletoned subgroup. After six months military service on the other hand, the short, light-skeletoned men had a higher relative PWC than the other subgroups. The difference was significant in relation to the tall heavy-skeletoned men. Lastly the highest values for shoulder thrust were found in tall heavy-skeletoned conscripts both on induction and after six months military service. At each of the two examinations the difference was significant in relation to tall light-skeletoned and short, light skeletoned conscripts.

In order to ascertain whether tall, heavy-skeletoned conscripts in the randomly chosen series had engaged in sport on a greater scale than the others or had done heavier work, all those who gave a record of competitive sports or heavy work were examined separately.

On induction, 27 per cent reported that they took part in competitive sports. These men had significantly higher PWC values than the others, from whom they did not differ however in height, femoral condylar breadth, skeletal quotient, or weight.—Furthermore, 15 per cent thought that their work as civilians had been heavy. Their PWC did not differ significantly from that of the others, though their muscular strength was significantly higher. Disparities also existed in body build: the conscripts who did heavy work were significantly shorter, had a greater femoral condylar breadth and a higher weight than the others. Of the conscripts randomly chosen six months after the start of military service 32 per cent stated that they had engaged in competitive sports and 19 per cent reported having done heavy work as civilians. Neither of these two categories, however, differed significantly from the other conscripts in PWC, muscular strength, or body build.

The series collected respectively on induction and after six months military service were also compared in regard to height, femoral condylar breadth, and skeletal quotient. The comparisons here were between corresponding skeletal-type subgroups and in no instance did significant differences emerge. The comparison was also extended to the previously reported conscripts who had been selected on registration. Here too

pressure the differences here being probably significant No other significant differences were noted between the two groups

The series collected at random on induction and after six months military service were, like the previously reported series classified according to skeletal build the four subgroups in each instance consisting of (1) tall heavy skeletoned (2) tall light-skeletoned (3) short, heavy skeletoned and (4) short light skeletoned conscripts

The values recorded in these four subgroups are presented in table 16 To clarify the differences between the series randomly collected on induction and after six months military service, in regard to PWC, relative PWC, and muscular strength, these factors are also illustrated in figure 4 The muscular strength is represented by shoulder thrust and the skinfold thickness by the values for region I as with the conscripts selected on registration In figure 4 the differences for the four subgroups are distinguished by broken lines, to signify that the two series are referable to different contingents of conscripts.

It is evident from table 16 and figure 4 that differences existed between the four subgroups in each series of randomly chosen conscripts,

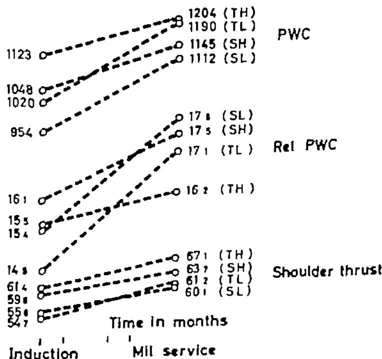


Figure 4
Conscripts di ided int four bgroups based n sk l tal build
TH = Tall hea y k l toned TL = T ll light sk let ned
SH = Sh t heavy k let ed SL = Short light-sk l toned

tion of the series at registration was probably tantamount to selection, though the degree of selectivity does not appear to have been very marked. Aside from the above-mentioned discrepancies no significant differences emerged between the series collected on varying bases

The conscripts selected on registration showed significant increases in height both between registrati n and induction and between induction and a point three months later the increase being most pronounced during the former interval Moreover the increase in height during military service was more marked in those conscripts who had been called up during the year immediately following regist ration than in those inducted one year later These findings suggest that the conscript has attained his full height approximately two years fter the year of registration — an assumption which is supported by the finding of Linroth (1957) and St lz & St lz (1951)

In th series collected on regist ration th weight rose significantly between regi tr ti n and induction but not sig ific ntly during the first three m nths of military service The ncrease between registration and i duct n was largely referable to those conscript who were not called p until th seco d year after registration. Those inducted during the first year after registration sh wed at the last examination during military service an increase f 1.0 kg as fr m the time of induction Here the increase in weight may have been a manifestation of normal growth.—For 103 conscripts who had completed eight months military service with I 1 in 1952—1953, Linroth (1957) found only a probably significant increase of 0 4 kg in weight. A possible explanation is that these conscripts, being 21 years old, had more closely approached the weight of fully grown adults than had those in the present investigation

The series collected at registration exhibited significant rises in PWC both between registration and induction and between induction and a point three months later The PWC was approximately equal for those called up during the first year after r gistration and those inducted one year later The value, moreover did not differ significantly from that btained for adult males in the City of Stockholm Health Survey of 1954 These findings suggest that the conscript had attained, on induction the average physical working capacity of adult males in Stockholm.

It was also found that the PWC did not increase after three months military service. This finding indicates that the physical demands of military service were readily met.

The PWC increased significantly as from induction until the completion of three months military service Those with the greatest increases did not, however differ significantly in body build from those with the smallest increases. The corollary is that the magnitude of the

there were no significant differences in height femoral condylar breadth or skeletal quotient on comparison of the respective series.

The coefficients of correlation between PWC on the one hand on the other heart volume, height, femoral condylar breadth skeletal quotient and weight were determined in the two randomly collected series The results are presented in table 17 The highest coefficient of correlation was that between heart volume and PWC *both in those studied* on induction and in those examined after six months military service The standard error of estimate was 167 kpm/min on induction and 154 kpm/min after six months.

Also determined were the coefficients of correlation between shoulder thrust on the one hand and height femoral condylar breadth skeletal quotient, and weight on the other Table 18 shows the results Both on induction and after six months the highest coefficient of correlation was that between body weight and shoulder thrust. The standard error of estimate amounted to 9 6 kp on induction and 8 0 kp after six months military service

4. Discussion of Results

The assumption that the conscripts *who had been examined on registration*, on induction, and after three *months military service* were genuinely representative is largely supported by the results obtained in the randomly collected series For examinations at corresponding times, *significant differences* between the series were exceedingly rare The observation of a *significantly* lower value for shoulder thrust in the conscripts *randomly collected on induction* suggest that the groups chosen for examination at the *time of registration* may have constituted a selection of physically strong individuals

Significant differences between those selected on registration and those collected at random were also noted during military service The randomly chosen groups had for instance significantly lower values for skinfold thickness. The difference between the two series may have been due to the fact that the randomly selected conscripts had been longer in military service

Disparities also emerged at the examination done in connection with induction For instance, the PWC values of the randomly chosen conscripts were higher for those who had engaged in competitive sports. No such inequality was recorded for the conscripts selected in connection with *registration* Of the randomly chosen conscripts examined on in*duction* those who did heavy work differed both in muscular strength and in body build from the others Here too, no corresponding difference was found for the men selected at registration The results for the randomly chosen conscripts must be considered in this respect more representative than the findings for the others.

The procedure followed in collec

B Naval Cadets

Since it was considered worthwhile to compare PWC, muscular strength, and body build of conscripts with the corresponding factors in another group of approximately the same age a number of naval cadets at KSS was similarly tudied The initial examination were made in connection with the ptitude tests for KSS in April and May 1959 Concurrently with these tests the prospective naval cadets were also stu dying for their matriculation examinati ns.

Th cadets were subjected to further investigation in the month of August after three months training On each occasion 63 cadets 20 years of age were examined

Table 19 shows the results obtained on the two occasions as well the differences between the means. At the time of the aptitude tests th I WC ue wa lower than that of th conscript on induction though the difference was not significant. Th alues for muscular strength were approximately equal to those for the series chosen t random on nduction xcept in the case of shoulder thru t for which the na al cadet had significantly higher alue

The heights of the naval cadets nd the conscripts were also compared the cad t being taller Their height differed sig ifica tly from that of th conscripts on registration.

Following the summer training th n al cadet exhibited significant increases in PWC, relative PWC, and heart volume. Higher va as lues were also obtained for all muscular strength determinations, though here significant differences were f und only for shoulder thrust Significantly higher weights and heights were noted after the period of training, while significantly lower values emerged for systolic and diastolic blood pressures. Further more, the skinfold thickness decreased in all regions, the difference being significant in region III, probably significant in region II, but not significant in region I Lastly the resting pulse was lower after the summer training, the difference being probably significant

For the naval cadets the PWC value in August after three months physical training was lower than that for the conscripts after a cor responding training period, though the difference was not significant. At the same time all muscular strength values in the naval cadets were higher than those recorded in the randomly chosen conscripts *after* six months military training The difference was significant only *for* houlder thru t.

Anoth er question studied w s whether those cadets who showed an increase of I WC differed from the others in body build The findings are presented in table 20 Of the total of 63 cadets, 27 (43 per cent) showed PWC increase of more than 100 kpm/min while 21 (33 per cent) *exhibited* an improvement *of* less than 100 kpm min. Those with

initial PWC value is of greater relevance than the skeletal build for the increase in PWC. The results thus suggest that the augmentation of PWC was not correlated to body build A study of the four subgroups with different skeletal types showed on the other hand, that the PWC differences between them had become less pronounced conscripts with the lowest initial values, i e., those of short stature having exhibited the greatest increase —Individuals of varying build thus seem to approach during military service one and the same PWC value —The results for the conscripts randomly chosen on induction were similar to those for the series collected after six months military service

In the series collected on registration the relative PWC also increased significantly during military service The findings indicated futhermore that the differentiation between the subgroups with differing skeletal characteristics was more pronounced after three months military service than it had been on induction

Turning to the muscular strength the values in the randomly collected series were higher for those examined after six months military service than for those tested on induction The difference was significant however only in respect of shoulder thrust In the series selected on registration the values for muscular strength were also higher during military service than on induction though here no significant differences were found —A moderate increase in muscular strength thus seems to have occurred during military service As regards the muscular strength in the randomly chosen conscripts the tall heavy skeletoned subgroup had the highest values both on induction and during military service Approximately the same differences emerged between this subgroup and the other three subgroups at both examinations.

The coefficient of correlation between PWC on the one hand and on the other heart volume height femoral condylar breadth skeletal quotient and weight was highest in respect of heart volume both on induction and after military service The coefficient for the heart volume was not however high enough to warrant consideration as a criterion of the physical working capacity With a standard error of estimate ranging from 150 to 170 kpm/min the PWC value deduced from the heart volume may vary by several hundred kpm/min a variation which must be considered excessive — It would be equally unfeasible to take individual body weights in randomly collected series as a criterion of muscular strength On the other hand it might well be feasible to group such series roughly on the basis of heart volume for estimation of PWC, and on the basis of body weight for estimation of muscular strength These findings are on the whole consistent with those reported by Linroth (1957) in the case of conscripts examined in 1952 and 1953

B Naval Cadets

Since it was considered worthwhile to compare PWC, muscular strength, and body build of conscripts with the corresponding factors in another group of approximately the same age a number of naval cadets at KSS was similarly studied. The in itial examinations were made in connection with the aptitude tests for KSS in April and May 1959 Concurrently with these tests the prospective naval cadets were also stu dying f their matriculation examin tions.

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The heights of th naval cadets nd the co scripts were also compared the cadets being taller Their I ight differed significantly from that f the conscripts on registration

Followi g the ummer training th naval cad t exhibited signifi cant increases in PWC, relative I WC, and heart volume. Higher va as lues were also obtained for all muscular strength determinations though here significant differences were found only for shoulder thrust Significantly higher weights and heights were noted after the period of training, while significantly lower values emerged for systolic and diastolic blood pressures. Further more the skinfold thickness decreased in all regions, the difference being significant in region III, probably significant in region II but not significant in region I Lastly the resting pulse was lower after the summer training, the difference bei g probably significant.

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Another question tudied was wh ther those cadet who showed an increase f I WC differed from the others in body build The findings are presented in table 20 Of the total of 63 cadets, 27 (43 per cent) showed a PWC increase of more than 100 kpm/min, while 21 (33 per cent) exhibited an improvement of less than 100 kpm/min. Those with

the greatest increases had the lowest initial values.—With regard to height and *femoral condylar breadth* no significant difference existed be *tween those* with the smallest and those with the greatest increases As respects weight, on the other hand those whose PWC improved most showed a lower value the difference being probably significant.

Similarly it was sought to ascer tain whether those with the greatest increases in muscular strength dif fered from the others The shoulder thrust rose by more than 5 kp in 28 of the 63 cadets (44 per cent) and by less than 5 kp in 26 (41 per cent) *The results are presented in table 21* Those with the greatest increase had the lowest initial values for shoulder thrust though not significantly lower than the values for those with the smallest increase Nor in regard to height femoral condylar breadth weight or PWC did any significant difference emerge between those with the greatest and those with the least increase of muscular strength

The naval cadets, like the con scripts, were also grouped with respect to skeletal build the four subgroups comprising (1) tall *heavy skeletoned* (2) tall light skeletoned (3) short heavy skeletoned and (4) short light-skeletoned cadets Table 22 presents the data recorded in each of these four subgroups at the time of the aptitude tests as well as three months later The skinfold thickness is tabulated only for region III where a signifi cant decrease was found The mus*cular strength* values refer only to shoulder thrust since the methodologic error in determination of that factor was less than those for other muscular strength factors. In addi tion the behavior in respect to PWC, relative PWC, and shoulder thrust is illustrated in figure 5

It will be seen from the table and figure that at the aptitude tests short, light-skeletoned naval cadets had the highest PWC value and short heavy skeletoned cadets the lowest. The difference between the subgroups was in no case significant. *After three months training tall* heavy-skeletoned cadets had the *highest and short* heavy skeletoned cadets the lowest PWC value the differences between the various subgroups were not, however signifi cant The increase in PWC during training was greatest in the tall heavy skeletoned and least in the short light skeletoned cadets

At the time of the aptitude tests the highest value for relative PWC was found in the short light skeletoned subgroup and the lowest value in the tall heavy-skeletoned subgroup, the difference being signifi cant The value continued to be highest in the former subgroup after the summer training By *this time however the differences between* this and the other subgroups were *in no case significant*

Both at the aptitude tests and after *the summer training the tall heavy* skeletoned subgroup exhibited the *highest shoulder thrust* value as will be seen from table 22 and figure 5

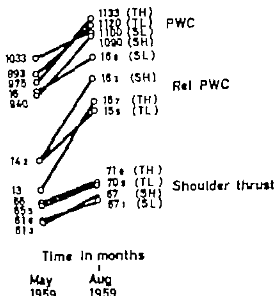


Figure 5

Naval cadets divided into four subgroups based on skeletal build
TH = Tall, heavy-skeletoned; TL = Tall, light-skeletoned
SH = Short, heavy-skeletoned SL = Short light-skeletoned

The lowest values were found on both occasions in the short cadets. None of the differences between the subgroups, however were significant

Those cadets who engaged in competitive sports were examined separately and compared with the others in order to ascertain if they fell into any particular category of skeletal build Of the 63 cadets 20 (32 per cent) stated, at the time of the aptitude tests, that they engaged in competitive sports. They did not, however, diverge from the others in respect to height, femoral condylar breadth skeletal quotient, weight, PWC, or shoulder thrust. Although their PWC increased significantly during training, it did not reach a significantly higher level than that recorded in the other cadets

The coefficients of correlation between PWC on the one hand and, on the other heart volume height, femoral condylar breadth, skeletal quotient and weight were determined both in connection with the aptitude tests and after three months training The results are set forth in table 23 On each occasion the highest coefficient of correlation

was that for the heart volume The standard error of estimate amounted to 171 kpm/min and 170 kpm/min respectively

The corresponding coefficients between on the one hand shoulder *thrust and* on the other height, femoral condylar breadth skeletal quotient and weight were also determined the resulting data being presented in table 24 The highest coefficient was that for weight both initially and after three months training On both occasions the standard error of estimate amounted to 7 9 kp

Discussion—The height of these *naval cadets increased significantly* during the three months of training This finding indicates that students 20 years old have not attained their full adult stature

The weight too increased significantly as from the aptitude tests until a point three months later This rise may have been attributable to continuing growth The skinfold thickness decreased during the summer training a circumstance which could have resulted in weight loss rather than gain During the same period the muscular strength on the *other hand underwent an increase* possibly due to an augmentation of muscle *tissue which in turn may* have contributed to the gain in body weight

The naval cadets at the time of the aptitude tests had a lower PWC than did the conscripts on induction Although the difference between these series was not significant *it may indicate that the* cadets had led a more sedentary life for some time prior to the examinations than the conscripts This is suggested by the cadets matriculation studies in connection with the aptitude tests, and by the fact that the PWC of the athletic cadets did not differ from that of the others. This might explain not only the low PWC values in the cadets but also the fact that the four subgroups exhibited only slight disparities in PWC, no significant differences being found either at the aptitude tests or after three months training

Further those cadets with the *greatest increase in PWC did not* significantly diverge in body build *from the others The PWC increased* most however in the subgroup of tall heavy skeletoned cadets Hence the interrelationship of the four subgroups was similar on the whole to that of the previously reported conscripts

The relative PWC rose significantly in the cadets during the three months of training The four subgroups presented certain disparities at the time of the aptitude tests, the highest value being found in the short, light skeletoned cadets, whose relative PWC differed significantly *from that of the tall, heavy-skeletoned* subgroup These disparities, *moreover were more* pronounced than those found in the conscripts *on induction—a circumstance* possibly attributable to greater physical inactivity *in the cadets After* three months training the relative PWC

was still highest in the short, light skeletoned subgroup of cadets, but did not differ significantly from the corresponding values in the remaining subgroups

During the three months training period the muscular strength in creased the rise being significant only for shoulder thrust. Both at the aptitude tests and three months later the highest value for shoulder thrust was recorded in the tall heavy-skeletoned subgroup in no in stance however did the figures dif fer sig ificantly from those in the ther three ubgroups. Furthermore, those with the greatest increase in muscular strength did not differ from the others in body build.—Of the conscripts chosen at random on induction and after six months mi litary service the tall, heavy skeletoned subgroup had likewise shown the highest value for shoulder thru t, the differences between that subgroup nd both the tall, light-skele- t ned and th sh rt, light-skeletoned subgroups being significant.—The disparities between bgroups were accordingly m re pronounced in co script than in na l cadets, possibly because the latter on the whol had led a more sedentary life

The coefficient f correlation between PWC on th on hand and, n the other heart olum height, femoral condylar breadth skeletal qu tient, nd weight was highest for heart vol m both t the p- titud test and three mo ths later The t ndard error of estimate was 171 and 170 kpm min respectiv ly The correlation therefore, as in the case of the conscripts, was not such as t permit reliable estimation of physical working capacity on the basis of heart volume. The pos sibilities of evaluating muscular strength on the basis of body weight were similar for the cadets and the conscripts.

C Trainees at the Swedish Forces Physical Training School (GIS)

It was also deemed worth while to tudy th PWC and muscular strength in relation to certain types of physique in ubjects whose work ing capacity and muscular strength should logically be superior to those f conscripts and naval cadets. GIS trainees were assumed to satisfy these desiderata A series of them was therefore tudied before the start of training course well as e month and four months later The course wa opened in October and concluded the following March, the last three weeks being devoted to training in the mountains, including ski exercises. The series comp ised 48 officers and warrant fficers with a mean age of 30 (range 24—40) The m jority of them had prior to the course engaged in athletic acti ities for periods varying from four to more than ten years.

The means for all subjects at the three examinations re listed in table 25 Even at the start of training the PWC exceeded the highest

average values for the conscripts and the naval cadets It was also higher than the PWC of 1185 kpm/min determined by Holmgren *et al* (1957) in normal men with a mean age of 28 (range 21—40)

As regards muscular strength the values determined at the start of the course accorded on the whole with those observed in the naval cadets after three months training The results obtained after one month s and four months training are, in table 26 compared with those recorded at the opening of the course. At the second examination the PWC, heart volume, and relative PWC had increased the difference being probably significant for the first mentioned and significant for the two last mentioned factors In addition the resting pulse and the muscular strength in the left hand were lower the differences being probably significant. Otherwise no significant differences emerged

Between the values recorded at the first and last examinations on the other hand a number of significant differences were found The PWC, heart volume, and relative PWC were significantly higher after four months training At the same time all muscular strength values were higher the difference being probably significant for shoulder pull Furthermore significantly lower values were noted for weight as well as for skinfold thickness in regions II and III while the lower value for skinfold thickness in region I was probably significant. Finally a significantly lower resting pulse was recorded at the conclusion of training

The trainees at GIS were like the conscripts and naval cadets, grouped with respect to the greatest and the smallest increase of physical working capacity the two groups then being compared It was found that 17 (39 per cent) of the 44 trainees had at the last examination, increased their PWC by more than 200 kpm/min as compared with the initial values Fifteen (32 per cent) showed an increase of less than 200 kpm/min. Table 27 presents the results recorded for these two groups at the initial examination They exhibited no significant differences in height, femoral condylar breadth or weight. However those who showed the greatest increase in PWC had the lowest initial values for both absolute and relative PWC, the differences being probably significant.

Between the first and last examinations the muscular strength expressed as shoulder thrust, was found to have increased in 17 of the 44 trainees (39 per cent) and to have decreased in 18 (41 per cent) The results for these two groups are reported in table 28 As regards height, femoral condylar breadth weight and PWC no significant differences were found between the two groups. Trainees with the greatest increase of muscular strength expressed as shoulder thrust had the lowest initial value which however did not differ significantly from the corresponding value for those whose muscular strength decreased

This particular series was consi

dered too small for classification into four subgroups as before instead two subgroups were formed on the basis of skeletal build. One of these subgroups comprised trainees with a skeletal quotient below the mean, i.e. those with a heavy skeleton in relation to height the other trainees with a skeletal quotient exceeding the mean, or those with a light skeleton in relation to height. The results obtained in the two subgroups at the three examinations were compared The muscular strength was recorded only in respect of shoulder thrust, the methodologic error being least for the muscle group concerned The skinfold thickness is tabulated only for region III in which the decrease as between the initial and the final examination was most pronounced. The values obtained are set forth in table 29 from which it is evident that the fall in body weight was largely referable to the trainees with a high skeletal quotient. This subgroup, moreover had a lower shoulder thrust value at the last than at the first examination whereas the reverse was true of the subgroup with a low skeletal quoti nt Th changes, however w re n t significant The skinfold thicknes d creased more in those with a low than in those with a high keletal quotient

Data n PWC, shoulder thrust, and relati PWC are presented in figure 6 It will be seen that the PWC was greater for those with a low than for those with a high keletal quotient, both at the start and on completion of the training course. The differences were significant.— The subgroup with a low skeletal quotient also had the highest value for relative PWC throughout the training course though at no time was the difference significant. Those with a high skeletal quotient showed, howeve the greatest increase.—As respects, lastly shoulder thrust, the highest value throughout the course was observed in those with a low skeletal quotient. Here too the dif ferences were in no case significant.

Comparisons were also made of respectively the GIS trainees, the conscripts selected on registration, and the naval cadets after each of the three categories had been broken down into a group with a high and a group with a low skeletal quotient. For this purpose the tall, heavy skeletoned, and the short heavy skeletoned subgroups were in each instance combined to form a group with a low skeletal quotient for each of the series In the same way the tall, light-skeletoned and the short, light-skeletoned subgroups were combined to form a group with a high keletal quotient The results which emerged in respect of the conscripts and the naval cadets, grouped as above are set out in table 30 The PWC and relati e PWC values are also illustrated in figure 7

The comparable groups of conscripts and naval cadets showed no significant differences in height, femoral condyl r breadth weight, and PWC at corresponding examinations. A study of figure 7 shows,

average values for the conscripts and the naval cadets It was also higher than the PWC of 1185 kpm/min determined by Holmgren *et al* (1957) in normal men with a mean age of 28 (range 21—40)

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This particular series was consi

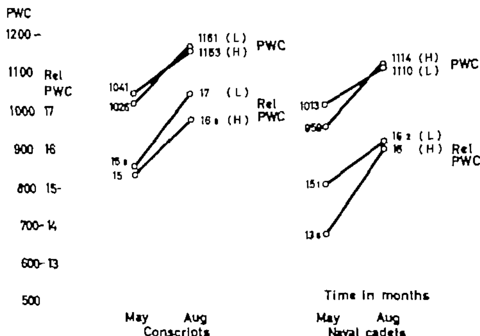


Figure 7

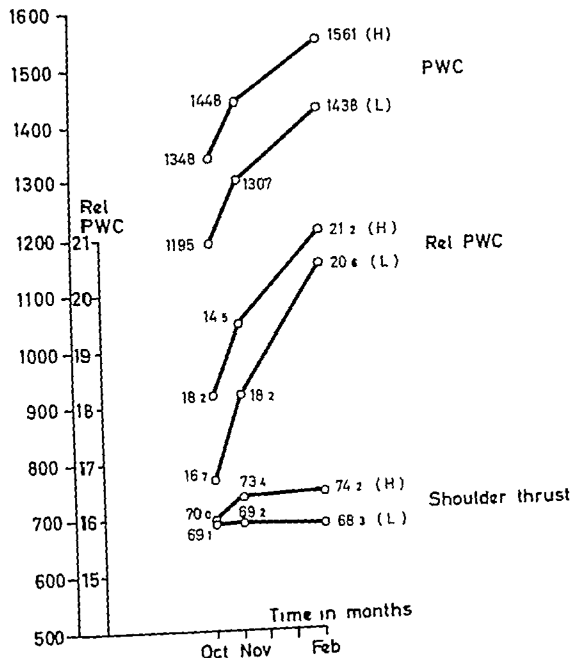
Comparison f conscript nd naval cad ts classified in two groups on the basis f sk let l b ild

H = Hea y skeleton; L = Light skeleton

however that the course in the conscripts differed somewhat from that in th naval cadet the conscripts with a high skel tal quotient exhi b ted th greatest augmentation of PWC, but lso h wed increased dif ferentiati of the rel tive PWC lues. Of th na al cadets, n the th r hand, those with a low keletal qu tient howed th greatest in rease n PWC wh reas the differ ntiation f th relative PWC values becam les pron need A regards th PWC no ignificant differences were f und, t corresponding exami nati s, between those with high nd those with low keletal quotients the respective series.

The post training results in the respective low-skeletal-quotient subgroups of GIS trainees, naval cad ts, a d conscript selected t regist ration w re lso compared. No signi ficant differences in height and femoral condylar breadth were found between the three categories. The GIS trai ees had however a signi ficantly higher PWC than the other two categories, as well as higher shoulde -thrust values, for which the difference was significant in relation to the conscripts and probably sig ificant in relation to the naval cadets.

Th corresponding subgroups with a high skeletal quotient were like-



Figu [illegible]

GIS train es lassif d in two groups n th basis f k l l l bu ld
H = He g k l l n L = Light sk l l n

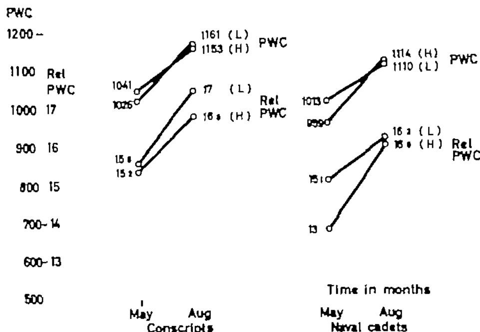


Figure 7
Comparison of conscripts and naval cadets classified in two groups on the basis of skeletal build
H = Heavy skeleton; L = Light skeleton

h wever that the course in the con scripts differed somewhat from that in the naval cadets the conscripts with a high skeletal qu tient exhi bited th greatest augmentation of PWC, but also showed increased dif ferentiation of the relati e PWC values. Of the naval cad ts, on the other hand, th se with a low skeletal qu ti nt showed th greatest increase i PWC, whereas the differ entiation of the relative PWC values became less pronounced. As regards the PWC n gnific nt differences were f und, t corresponding examinati ns, between those with high and those with low keletal qu tients in th respective series.

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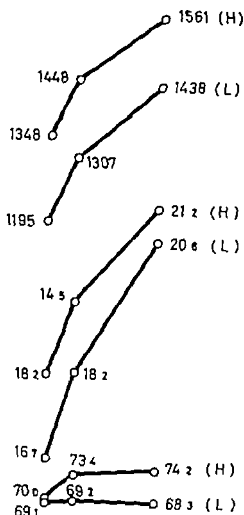


Figure 6

GIS trainees classified in two groups on th ba is f skel t l bu ld
H = H a g k let n L = Light skel t

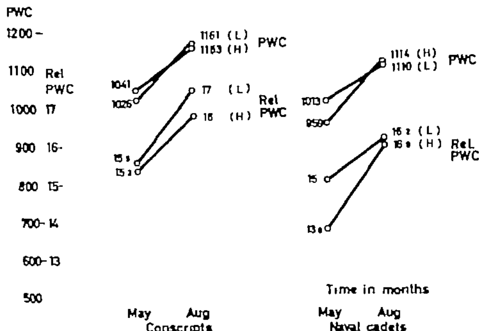


Figure 7

Comparison of conscripts and naval cadet classified in two groups on the basis of sk letal build

H = Heavy skeleton L = Light skeleton

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The corresponding subgroups with a high skeletal quotient were like-

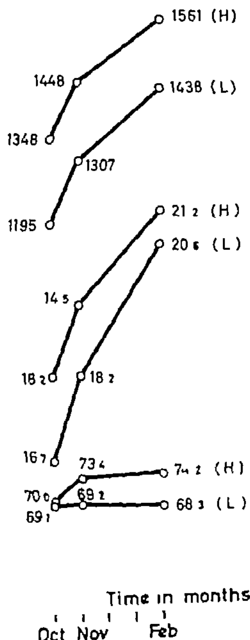


Figure [illegible]

GIS tra nees classified in two groups n th bas f Lel tal build
H = Heavy skel ton L = Light k l t n

and the low-skeletal-quotient subgroups was observed in the naval cadets and the conscripts. Moreover the GIS trainees with low and high skeletal quotients respectively showed higher PWC values than did the corresponding categories of cadets and conscripts These disparities may have been attributable to a number of different factors—e.g. possible differences in nature and intensity of the physical training also the previous athletic activity of the GIS trainees as well as the age differences between the series.

The relative PWC increased significantly for the GIS trainees during the f ur months training period. The values were higher for those with a low than fo those with a high skeletal quotient. The diff ence between the two subgroups—which was at no tim significant—decreased during the course of training. F those with a high skel tal quotient th high relative PWC was attributabl both t a decrease of body weight and to an increase in PWC, whereas fo those with a low keletal qu ti nt it was referable solely to an increase in PWC. Thus the physical training seems to have entailed equally high relative PWC alues fo GIS trainees irrespective f kel tal build.

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As with the conscripts and cadets, the coefficient of correlation be tween PWC on the one hand and heart volume height, femoral condylar breadth, skeletal quotient and weight was highest for heart volume at all three examinations of the GIS trainees. The standard error of esti mat was 187 161 and 170 kpm/min respectively Not even for the GIS trainees, therefore, was the correlation so pronounced as to afford a possibility of estimating the PWC with any degree of accuracy from the heart volume. As respects esti mation of muscular strength on the basis of body weight or femoral condylar breadth, the overall picture in the GIS trainees was similar to that in th naval cad ts and the con scripts.

D Weight Lifters, Wrestlers and Runners

In the preceding series the types of physical activity or training pur sued were not necessarily adapted to

wise compared following the relevant training periods The naval cadets were found to be significantly taller than the CIS trainees but otherwise no significant differences either in height or femoral condylar breadth were observed between the three subgroups. For those with a high as for those with a low skeletal quotient the PWC value was significantly higher in GIS trainees than in the two other series

The coefficients of correlation between PWC on the one hand and on the other hearth volume, height femoral condylar breadth skeletal quotient and weight were determined at the start of the course and after one and four months training The results are presented in table 31 At each of the three examinations the highest correlation coefficient was that between heart volume and PWC. The standard error of estimate was 187 161 and 170 kpm/min respectively

Also calculated were the coefficients of correlation between shoulder thrust on the one hand and height, femoral condylar breadth skeletal quotient and weight on the other These results are shown in table 32 At the initial examination the highest coefficient was that between weight and shoulder thrust After both one month s and four months training the coefficient for femoral condylar breadth and shoulder thrust was higher than that for weight and shoulder thrust

Discussion —No significant change of height occurred during the four months training period This circumstance reflects the fact that the subjects in this series had already attained their full adult height

The body weight fell significantly during the four months period concomitantly with significant reductions of skinfold thickness which may have contributed to the fall Although all muscular strength values rose the increase was probably significant only in respect of shoulder pull Since the muscular strength had not increased to a major degree it must be assumed that the amount and weight of muscle tissue had not risen appreciably The body weight therefore is unlikely to have been influenced by changes in the amount and weight of muscle tissue

Although the PWC values of the CIS trainees were relatively high at the start of the course they nevertheless rose fairly rapidly during training A possible explanation is that the duties of many of the trainees had previously been more physically demanding than they were at the start of the course

Those whose PWC increased most during the four months training did not differ in body build from those whose PWC increased least The former category however had the lowest PWC value at the start of the course It would seem therefore that the increase in PWC during a physical training course is dependent more upon the initial PWC than upon the skeletal build

As regards the absolute PWC no similar differentiation of the high

and the low-skeletal-quotient subgroups was observed in the naval cadets and the conscripts. Moreover the GIS trainees with low and high skeletal quotients respectively showed higher PWC values than did the corresponding categories of cadets and conscripts. These disparities may have been attributable to a number of different factors—e.g. possible differences in nature and intensity of the physical training also the previous athletic activity of the CIS trainees as well as the age differences between the series.

The relative PWC increased significantly for the CIS trainees during the four month training period The values were higher for those with a low than for those with a high skeletal quotient The difference between the two subgroups—which was at no time significant—decreased during the course of training. For those with a high skeletal quotient the high relative PWC was attributable both to a decrease of body weight and to an increase in PWC, whereas for those with a low skeletal quotient it was referable solely to an increase in PWC. Thus the physical training seems to have entailed equally high relative PWC values for GIS trainees irrespective of skeletal build.

With regard to the muscular strength, higher values were noted after the four months training period though the increases were relatively insignificant. Only for shoulder pull was a probably significant difference found. The muscular strength values, however were comparatively high even at the initial examination—a circumstance which may have been attributable to previous physical exercise The fact that the muscular strength notwithstanding the marked augmentation of PWC, showed no major increase in the CIS trainees may be considered to substantiate the assumption that the initial muscular strength sufficed to meet the subsequent physical demands

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D Weight Lifters, Wrestlers and Runners

In the preceding series the types of physical activity or training pursued were not necessarily adapted to

individual physiques Hence an investigation of various types of athletes was undertaken It may be *assumed that an athlete will be* attracted to that or those branches of athletics which would seem to offer him the greatest opportunity to excel The result will be a natural selection of athletes in the different fields of sport based not only on physique but also on innate ability

In this investigation two different *forms of physical training were* studied in three different types of athletes—weight lifters, wrestlers and runners The weight lifters and wrestlers were assumed to have pursued a form of athletics which increased principally the muscular strength and the runners a form which had augmented in particular the physical working capacity

The weight lifters and wrestlers together formed a group of 48 athletes with a mean age of 26 (range 20—34) The other group consisted of 28 middle-distance runners with a mean age of 26 (range 20—36) Further data on these athletes will be found in Chapter II The means and their standard deviations for each of the two groups are set forth in table 33

For the weight lifters and wrestlers (table 33) the mean height was found to be less than that in any of the preceding series the differences in each instance being significant The discrepancy was least pronounced in comparison with the GIS trainees and the conscripts. The weight lifters and wrestlers also had significantly lower skeletal quotients than any of the other series Moreover they were heavier their *body weight differed significantly* from that of the conscripts and naval cadets but not from that of the CIS trainees. Lastly they had significantly higher values for muscular strength than any of the other series though their PWC approximated that of the CIS trainees at initial examination

The runners (table 33) showed no significant divergence in height from any of the preceding series. However they had higher values for the skeletal quotient the differences here being significant except in comparison with the naval cadets. Further the runners body weights were lower than those in any of the preceding series The difference was least in comparison with the conscripts and significant only in comparison with the CIS trainees The runners lastly had a higher PWC as well as a higher relative PWC than any of the other series Their muscular strength however showed *no major deviation either from that* of the conscripts during military service or from that of the naval cadets at the time of the aptitude tests.

Substantial discrepancies were found between the weight lifters and wrestlers on the one hand and the runners on the other (table 33) in regard to height femoral condylar breadth skeletal quotient absolute and relative PWC, *and muscular* strength Further the standard de-

viations were almost invariably greater for the weight lifters and wrestlers. Some findings in the run ners were also inconsistent with values recorded in the literature. For instance both the total hemoglobin per kilogram of body weight and the blood volume per kilogram of body weight were significantly higher than the figures previously reported for healthy men (Kjellberg et al 1949 Holmgren et al., 1957 1958) The runners, moreover had significantly higher PWC values than those determined by Holmgren et al (1957) for ten racing cyclists with a mean age of 20 (range 18—31) In addition to these differences, the runners also had a significantly greater heart volume per kilogram of body weight and a significantly lower hemoglobin concentration than the weight lifters and wrestlers.

The relationship of heart volume and total hemoglobin to PWC in the runners also deviated from that customarily found When the values were subjected to comparative study with the use of regression analyses devised at Karolinska Sjukhuset (Kjellberg et al 1949 1951) the PWC was found to be great in relation both to the heart volume (+ 23 per cent) and to the total hemoglobin (+ 19 per cent) the differences being significant The corresponding relationship for the weight lifters and the wrestlers did not deviate from the same regression line The results in both categories are illustrated in figure 8.

Further comparisons were made between the athletes and the GIS trainees For this purpose the weight lifters and wrestlers were compared with those of the GIS trainees who had a low skeletal quotient, and the runners with those who had a high skeletal quotient. The results are shown in table 34 In the first mentioned comparison no significant differences emerged as regards height, femoral condylar breadth or skeletal quotient The shoulder thrust value for the weight lifters and wrestlers, however was higher than that for the relevant subgroup of GIS trainees, though the difference was not significant As respects PWC, relative PWC, and heart volume the subgroup of GIS trainees exhibited significantly higher values than those of the weight lifters and wrestlers.

On comparison of the runners and GIS trainees with a high skeletal quotient, no significant difference was found in respect of height femoral condylar breadth and skeletal quotient. The runners had a lower shoulder thrust value, although the difference was not significant, and a lower body weight the difference here being probably significant. For PWC and relative PWC the runners showed significantly higher values than the pertinent subgroup of GIS trainees.

The coefficient of correlation between PWC on the one hand and heart volume, height, femoral condylar breadth, skeletal quotient, and weight were determined both for the weight lifters and wrestlers and for

individual physiques. Hence an investigation of various types of athletes was undertaken It may be assumed that an athlete will be attracted to that or those branches of athletics which would seem to offer him the greatest opportunity to excel The result will be a natural selection of athletes in the different fields of sport based not only on physique but also on innate ability

In this investigation two different forms of physical training were studied in three different types of athletes—weight lifters, wrestlers and runners The weight lifters and wrestlers were assumed to have pursued a form of athletics which increased principally the muscular strength and the runners a form which had augmented in particular the physical working capacity

The weight lifters and wrestlers together formed a group of 48 athletes with a mean age of 26 (range 20—34) The other group consisted of 28 middle-distance runners with a mean age of 26 (range 20—36) Further data on these athletes will be found in Chapter II The means and their standard deviations for each of the two groups are set forth in table 33

For the weight lifters and wrestlers (table 33) the mean height was found to be less than that in any of the preceding series the differences in each instance being significant The discrepancy was least pronounced in comparison with the GIS trainees and the conscripts The weight lifters and wrestlers also had significantly lower skeletal quotients than any of the other series. Moreover they were heavier their body weight differed significantly from that of the conscripts and naval cadets but not from that of the CIS trainees Lastly they had significantly higher values for muscular strength than any of the other series though their PWC approximated that of the CIS trainees at initial examination

The runners (table 33) showed no significant divergence in height from any of the preceding series. However they had higher values for the skeletal quotient the differences here being significant except in comparison with the naval cadets. Further the runners body weights were lower than those in any of the preceding series The difference was least in comparison with the conscripts and significant only in comparison with the GIS trainees. The runners, lastly had a higher PWC as well as a higher relative PWC than any of the other series. Their muscular strength however showed no major deviation either from that of the conscripts during military service or from that of the naval cadets at the time of the aptitude tests.

Substantial discrepancies were found between the weight lifters and wrestlers on the one hand and the runners on the other (table 33) in regard to height femoral condylar breadth skeletal quotient absolute and relative PWC, and muscular *strength Further* the standard de-

the runners The results are shown in table 35 For each of the two ca tegories the highest coefficient of correlation was that between PWC and heart volume The standard error of estimate was 102 and 162 kpm/min respectively

Also determined were the coeffi cients of correlation between shoulder thrust on the one hand and height, femoral condylar breadth skeletal quotient, and weight on the other The results are set forth in table 36. In the case of the weight lifters and wrestlers the highest cor relation coefficient was that for body weight. The standard error of esti mate was 9,0 kp For the runners the coefficient of correlation was highest for femoral condylar breadth though the correlation was not significant The standard error of esti mate here amounted to 9 9 kp

Discussion.—The weight lifters and wrestlers diverged from the con scripts as well as from the naval cadets and GIS trainees in respect to height, keletal quoti nt, and weight. They showed, for instance, greater body weights s well as heavier skeletons in elation to height. This circumstance may re flect a tendency to ngage in those branches of athletics for which thei physiques were best suited. The lesser height of the wrestlers and weight lifters may however be partly attributable to the fact that such athletes compete in different weight classes and thu constitute a special selection.—The runners, for their part, were found to differ from the conscripts, naval cadets, and GIS trainees principally in regard to skeletal quotient and weight they did not differ in height. Thus they had both lighter skeletons in relation to height, and lower body weights than any of the aforementioned series. This finding too might be ascribed to the selective tendency suggested above

The weight lifters and wrestlers, furthermore, showed higher values for muscular strength than the conscripts, naval cadets, and GIS trai nees, while the runners had higher PWC and relative PWC values than any of those groups. This finding may be attributable to the above-mentioned selective tendency in conjunction with specially directed forms of physical training and inherent aptitude of the athletes Since the training of the weight lifters and wrestlers was principally concerned with the muscular strength it is here designated as primary muscular exercise In the runners, on the other hand, the physical training had chiefly involved the circulatory organs, and hence is here termed primary circulatory exercise» In the previously described series the training may be said to have con stituted a mixture of these two forms of exercise. As regards the muscular strength, the values referable to the shoulder girdle and the hands were considered representative of the overall muscular strength, the muscels involved being unlikely to reflect directly the effects of the runners specialized training

Heart volume

1000

500

PWC kpm/min

500 1000 1500 2000

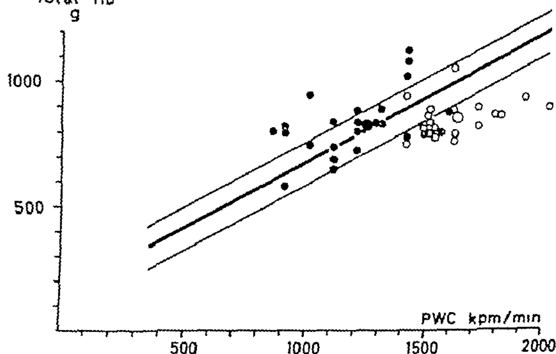


Figure 8

Physical working capacity in relation to total-Hb and heart volume

○ = Runners

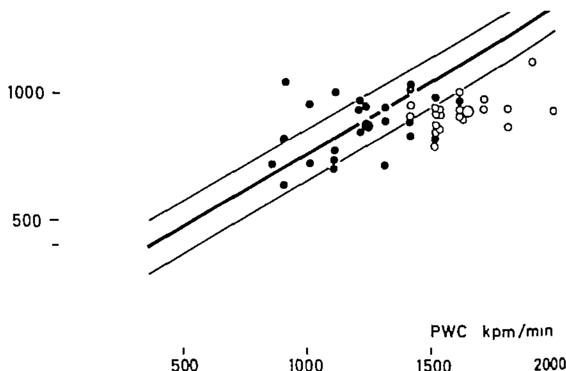
● = Weight lifters and wrestlers

the runners. The results are shown in table 35 For each of the two ca tegories the highest coefficient of correlation was that between PWC and heart volume The standard error of estimate was 192 and 102 kpm/min respectively

Also determined were the coefficients of correlation between shoulder thrust on the one hand and height, femoral condylar breadth, skeletal quotient, and weight on the other The results are set forth in table 36. In the case of the weight lifters and wrestlers the highest cor relation coefficient was that for body weight. The standard error of estimate was 9 0 kp For the runners the coefficient of correlation was highest for femoral condylar breadth though the correlation was not sig nificant. The standard error of esti mate here amounted to 9 9 kp

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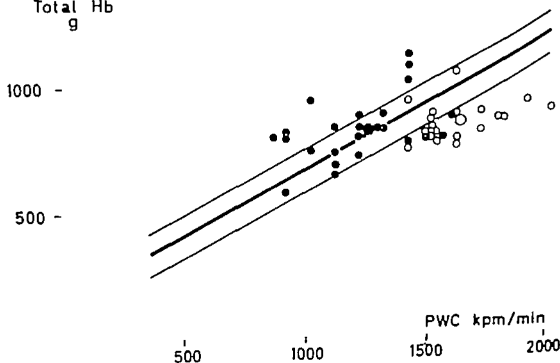


Fig re 8

Physical working capacity in rel ti n t t tal-Hb and hea t volum

○ = *Runn*

● = *W ight lift and w estl rs*

Chapte II

GENERAL DISCUSSION

In this investigation a number of problems relating to various body parameters and developmental conditions were studied, particularly with respect to conscripts, whose physical working capacity and muscular strength were followed as from registration until induction, as well as during military service, with the aim of elucidating the changes which those factors underwent. The PWC values determined at registration of conscripts for four consecutive years were also studied. In addition, certain physical parameters were correlated with the PWC and muscular strength in order to ascertain whether any data of value could be secured. The results for the conscripts were then compared with those obtained for other groups, some of which were largely similar to, nd others divergent from, the conscripts in respect to age, PWC and muscular strength. Another aim here was to ascertain if groups whose PWC and muscular strength values were higher than those f the conscripts differed from the latter in their responses to physical trai ning. Lastly a number of athletes were tested in order to study the effects of specialized training. For this purpose two groups with certain natural athletic propensities based on body build were studied and the results compared with those obtained for other groups.

The skeletal type was taken as a basis for subgrouping according to body build on the assumption that the skeleton is one somatic factor which, in adult males, undergoes very little alteration as compared with e.g. muscular development and thickness of subcutaneous fat. Certain skeletal dimensions—the body height and the breadth of the femoral condyle—were used as criteria. Also recorded was the quotient of body height and femoral condylar breadth, termed the skeletal quotient. On this basis it was possible to break down the groups into four categories, these subgroups being classed as (1) tall heavy-skeletoned, (2) short, heavy-skeletoned, (3) tall, light skeletoned and (4) short, light-skeletoned. Each of the heavy skeletoned subgroups had a low skeletal quotient, i.e. heavy skeletons in relation to height and conversely each of the light-skeletoned subgroups had a high skeletal quotient.

Four different series with divergent characteristics were investigated. They comprised (1) conscripts, (2) naval cadets, (3) offi

Comparison showed that the weight lifters and wrestlers did not differ significantly in body build from the subgroup of GIS trainees with a low skeletal quotient The weight lifters and wrestlers, however had higher values for muscular strength but lower values for absolute and relative PWC. Their physical working capacity moreover accorded on the whole with that found at initial examination of GIS trainees Hence, predominantly muscular training does not necessarily lead to an increase of the PWC as determined in this investigation

The comparison also showed that the runners body build did not diverge significantly from that of the subgroup of GIS trainees with a high skeletal quotient The runners, however exhibited lower values for muscular strength but higher values for absolute and relative PWC. It may be concluded therefore that exercise principally designed to increase the physical working capacity will not necessarily augment the muscular strength as determined in the present investigation

The weight lifters and wrestlers on the one hand and the runners on the other showed major disparities not only in body build but in absolute and relative PWC as well as in muscular strength These discrepancies may be attributable to the aforementioned selective tendency of athletes, in conjunction with specialized types of physical training as well as innate aptitude

The coefficient of correlation between PWC on the one hand and heart volume, height femoral condylar breadth skeletal quotient and weight was highest for heart volume both in the weight lifters and wrestlers and in the runners However the correlation was not so pronounced that the heart volume might be considered a sufficiently reliable criterion of the PWC in the individual case This conclusion accords with the observations in the previously described series Similar findings applied to the correlation between body weight and respectively femoral condylar breadth and shoulder thrust in the weight lifters and wrestlers and in the runners.

responsible for the weight gain. Lin roth (1937) too, found in his in vestigations of 1952—1953 that con scripts gained weight during mili tary service. So far as can be judged however this gain was less than that noted in the present investigation A possible explanation is that since Linroth s conscripts were 21 years old their rat of physical development was slower as is suggested by the fact that their height did not increase duri g military service

The GIS trainees lost weight during the four months of intensive physical training This weight loss could have been due to a decrease in the amount of fatty tissue, as seemed evident from the values for skinfold thickness.

The weight lifters nd wrestlers had the highest, and th runners the lowest, body weights of any of the series. This circumstance might be ascribed to selectivity in athletics as respects body build, in conjuncti n with the effects of the relevant forms f sport pursued by the two groups The weight lifters engaged primarily in muscula exercise, which had served to increase the amount and hence the weight of muscle tissue. For the runners, n the other hand the values f kinfold thickness were extremely low

The body weight thus seems to in rease s rule until the ag f 20 r 21 largely a a result of continuing growth. Physical training, it would appear may influence the weight in one direction or the other depending on the type of training and the composition of the group in question.

The average *physical working capacity* ranged from 881 kpm/min for the conscripts on registration in 1959 to 1607 kpm/min for the runners. Inter-group variability in PWC values was substantial

For the conscripts PWC values of around 900 kpm/min were recorded at the registrations of 1957—1960 Conscripts who were 18 years old at the time of registration showed an average PWC which did not differ significantly from that reported by Wahlund (1948) as the lower limit for healthy adult males. The PWC increased significantly for the conscripts between registration and induction values of 1033 and 1054 kpm/min respectively were found on induction for two entirely different series. No significant PWC difference existed between the men inducted the year after registration and those who were not called up until one year later The PWC on induction also agreed on the whole with that noted for middle-aged men (1050 kpm/min) in the City of Stockholm health survey of 1954 (Frisk *et al* 1959) For conscripts the PWC *thus* rose until the time of induction, the increase probably being largely a manifestation of normal growth. By the time they were called up, more ove the *conscripts had* pparently attained the average PWC for ordinary adult males in Stockholm.

The PWC for the conscripts also rose *significantly* during the first three months of military service, the

cers and warrant officers, (GIS trainees) and (4) athletes. The conscripts and naval cadets were chosen at random insofar as this was possible whereas the GIS trainees and the athletes constituted selections of men who actively participated in athletics The respective mean ages varied Conscripts examined in connection with registration for military service were only 18 years of age while those examined on induction were either half a year or one and a half years older depending on the time they were called up Further the naval cadets were aged 20 whereas the mean ages of the GIS trainees and the athletes were 30 and 26 respectively Common to the series was the fact that they had engaged in some form or other of physical exercise. The conscripts, naval cadets and GIS trainees were examined prior to and at varying intervals after physical training the athletes, only after several years intensive training in their respective branches of athletics

Results varied for the different series In some instances this was attributed to selectivity in others to age disparities and in still others to an effect of specialized physical training or to a combination of these factors

As regards the results of individual examinations, the *height* was found to increase both for conscripts *and for* naval cadets during their respective training periods The gain however was less pronounced for subjects who were nearing the age of 20 These findings may be viewed as an effect of continuing growth The results are largely in accord with those reported by Stolz & Stolz (1951) For the GIS trainees no increase of height was found Those of the athletes who engaged in weight lifting and wrestling were on the average shorter than any of the other series. This finding may be attributed largely to selectivity since these athletes compete in different weight classes

As regards *skeletal quotient* the athletes diverged from the other groups. The weight lifters and wrestlers had a lower and the runners a higher skeletal quotient than any of the remaining series. Of athletes, therefore weight lifters and wrestlers appear to have unusually heavy and runners unusually light skeletons in relation to height No significant differences emerged between the other series in respect to skeletal quotient

In the conscripts as well as in the naval cadets the *body weight* increased during the respective training periods *It was thought that in* both instances the gain may have been due partly to normal growth and partly to an augmentation of muscle tissue since not only did the *height increase* but the muscular strength as well The increase in muscular strength was probably attributable wholly or in part to the training Assuming the above mentioned hypothesis to be correct it is impossible to say definitely which of the two factors was chiefly

Neither in the conscripts and naval cadets nor in the GIS trainees were any major disparities in skeletal type and body weight found between those with the greatest and those with the least PWC increase during physical training. However those with the greatest increases had exhibited the lowest, and those with the smallest increases the highest, initial values. These findings accord in principle with those recorded by Linroth (1957) for conscripts examined in 1952—53.

The *muscular strength* was determined in the shoulder girdle and the hands. The lowest shoulder-thrust value—57.8 kp—was noted in the conscripts randomly chosen on induction in 1960 and the highest—76.9 kp—in the weight lifters and wrestlers.

For the conscripts, naval cadets, and GIS trainees the improvement in muscular strength during physical training was relatively modest, significant increases being exceptional. Although the GIS trainees had shown a greater rise in PWC than the other two groups, their increase in muscular strength was not more pronounced. This circumstance suggests that at the start of training the muscular strength in the respective groups was on the whole commensurate with the demands made by the training.

The muscular strength values were highest for the weight lifters and wrestlers—a group which had heavier skeletons in relation to height than any of the other series. It would thus seem that the highest muscular strength values are associated with a particular body build in conjunction with a specialized form of physical training. Since the training was, in the case of the weight lifters and wrestlers, chiefly directed towards development of the muscular strength it is here designated as primary muscular exercise.

The runners, as mentioned above, showed the highest PWC values. Their muscular strength values, however, were quite ordinary. Their shoulder thrust for instance was 63.6 kp which substantially agreed with that recorded at initial examination of the naval cadets. Development primarily of the physical working capacity therefore will not necessarily entail an increase of the muscular strength as determined in this investigation. The PWC value for weight lifters and wrestlers—1213 kpm/min—approximated those for the GIS trainees at initial examination. Development chiefly of the muscular strength thus does not necessarily serve to augment the physical working capacity as determined in this study.

Neither for the conscripts nor for the naval cadets and the GIS trainees were any major disparities in skeletal build and body weight observed between those with the greatest and those with the smallest increases in muscular strength during physical training. However those who showed the greatest increases had the lowest initial values, and vice versa. These results are con-

increase being equally pronounced in the case of those called up the year following registration and those inducted one year thereafter Two different series of conscripts in I 1 showed respectively PWC values of 1155 kpm/min after three months military service and 1162 kpm/min after six months. In the former group the PWC ceased to increase after the completion of three months service The physical working capacity thus appears to be commensurate with the demands made by military service The men in the present investigation had, during military service, a significantly lower PWC than that of Linroth s (1957) conscripts examined in 1952 and 1953 Linroth recorded a value of 1252 kpm/min for 94 conscripts in I 1 after eight months service in 1953 he also observed a decline in PWC during military service. The explanation of the discrepancy may lie partly in the fact that the PWC values for Linroth s conscripts at the initial examination in 1952 were higher than those for conscripts on induction in 1958 and 1959 and partly in the fact that his initial values were referable to determinations made during the first two months of military service.

For the naval cadets in this study the PWC rose from 985 kpm/min at the time of the KSS aptitude tests to 1112 kpm/min after three months training Comparison with the conscripts on induction showed a lower mean PWC for the naval cadets at the aptitude tests though the difference was not significant

As regards the GIS trainees the PWC at the start of the training course averaged 1273 kpm/min The corresponding value after four months training was 1502 kpm/min Thus, even though this group began their training with a higher PWC than did the conscripts and the naval cadets their PWC values increased more during the training period than did those for the other two groups. These disparities may have reflected the influence of several different factors, notably differences in age and types of sport pursued as well as the fact that the GIS trainees constituted a selection of men with special athletic leanings.

The highest PWC was noted in the runners who showed a mean of 1807 kpm/min As a result of their particular type of training, moreover they also had a high PWC in relation both to the heart volume (+ 23 per cent) and to the total hemoglobin (+ 18 per cent) The highest PWC values apparently are attained therefore in subjects with specific skeletal characteristics who have undergone specialized training Since the training of runners involves principally the circulatory organs, it is here termed »primary circulatory exercise — Thus the highest physical working capacity was found in a group which constituted a special selection with respect to body build and which had pursued a specialized form of training

grouping constitutes a classification with respect to suitability for certain types of manual work. If the requirements for a given type of manual work are known it should thus be possible in large measure, on the basis of the present results to select from a series of subjects with varying body builds those with the best physical prerequisites for the duties in question.

istent with the findings of Lindegård (1956) and probably reflect a general trend towards adaption to the demands of the work—a trend that would seem to lead to interindividual equalization.

For the groups of subjects with *differing skeletal types* disparate results were noted.

The short, light-skeletoned subgroups, both of conscripts and of naval cadets, were those with the highest relative PWC values. In view of those investigations which indicate that the physical working capacity is correlated to the circulatory capacity (Kjellberg *et al.* 1949, 1951), it may be surmised that the higher relative PWC of short, light-skeletoned subjects is due to a greater capacity of the circulatory organs in relation to the body weight. From the aforegoing it appears, therefore, that short, light-skeletoned persons would be particularly suited for types of work that call for a high relative physical working capacity.

It emerged, furthermore, that the tall, heavy-skeletoned subgroups, both of conscripts and of cadets, were those with the highest absolute PWC and muscular strength values. Therefore, tall heavy-skeletoned subjects in general are more suited than others for duties in which both physical working capacity and muscular strength are the primary considerations.

The runners had light skeletons in relation to height, and high PWC values, whereas the weight lifters and wrestlers had heavy skeletons in relation to height, and high muscular strength values. It could thus be asked whether these findings, together with the results for the subgroups of conscripts and naval cadets, do not justify the conclusion that persons with light skeletons in relation to height are better suited than others for duties which require both a high absolute PWC and a high relative PWC. Also open to question is whether, by the same token, individuals with heavy skeletons in relation to height are not better fitted than others for duties requiring in particular a high PWC as well as high muscular strength. Assuming all this to be so, persons with average skeletal quotients would seemingly be more suited than others when both high absolute and relative physical working capacities and high muscular strength are required.

The correlation between PWC on the one hand and heart volume, height, femoral condylar breadth, skeletal quotient, and weight on the other was in no instance close enough to provide a reliable criterion of the physical working capacity in the individual case. The same was true in regard to the possibility of evaluating the muscular strength of an individual on the basis of his body build. Subgrouping according to skeletal type as described in this paper does not enhance the possibility of assessing the individual physical working capacity and muscular strength. It is plausible to assume, on the other hand, that such sub-

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The correlation between PWC on the one hand and heart volume, height femoral condylar breadth, skeletal quotient and weight on the other was in no instance close enough to provide a reliable criterion of the physical working capacity in the individual case The *same* was *true* in regard to the possibility of evaluating the muscular strength *of* an individual on the basis of his body build Subgrouping according to skeletal type as described in this paper does not enhance the possibility of assessing *the individual* physical working capacity and muscular strength. It is plausible to assume on the other hand that such sub-

(18 years) until induction (19—20 years) and of 0.4 cm during the first three months of military service.—The naval cadets, too, showed a significant increase of 0.4 cm on completion of three months training

The conscripts and naval cadets also gained weight. A significant rise of 1.4 kg in the interval between registration and induction was noted for the conscripts, and a significant increase of 1.0 kg for the naval cadets at the end of three months training. The weight gains were attributed in part to continuation of normal growth. GIS trainees on the other hand, showed a significant decrease of 1.3 kg in body weight during their training period. This weight loss was considered to have been due to a decrease in the amount of fatty tissue, as reflected in th reduced skinfold thicknesses — The highest body weight alu was noted in the weight lifters and wrestlers, nd the lowest in the runners. The finding may be ttributable partly to the specialized types of physical traini g and partly to the individual s tendency to engage in a form of athletics for which his physique is suited.

Th weight lifters and wrestlers had a lower and the runners a high r skeletal quotient than any of the other groups. Thi observation i assumed to eflect individual selectivity in athletics with respect to physique

The PWC of the conscripts showed, in the interim between registration and induction, a significant rise which was presumptively connected with continuing growth. During the first three months of military service, however there was an even more pronounced increase which appeared to be largely a result of the physical training associated with military service. Subsequently the PWC rose no further presumably because by the end of three months it was adequate for the demands of military service

Although the PWC of the GIS trainees was, at the start of their training course, significantly higher than that of the conscripts on induction and of the naval cadets at the initial examination, it nevertheless rose significantly during the first month of training. The explanation of this finding may well lie in the fact that the GIS trainees constituted a selection of men with special athletic leanings.

For the conscripts, naval cadets and GIS trainees the muscular strength in general increased but little under physical training a significant increase was recorded only for certain muscles in a few subject groups. This result suggests that the muscular strength in the respective series at the outset of their physical training approximated that which the training required

The highest values for PWC (circulatory capacity) were noted in the runners, and the highest muscular strength values in the weight lifters and wrestlers. These observations were accordingly referable to two groups of athletes who constituted

Chapter I

SUMMARY AND CONCLUSIONS

All of the subjects in this investigation underwent determinations of the physical working capacity (PWC) muscular strength and body build. The PWC (constituting an index of the functional oxygen transporting capacity of the circulatory system) was determined on a bicycle ergometer *ad modum* Karolinska Sjukhuset the muscular strength by dynamometric recording and the thickness of the subcutaneous fat by measuring skin folds in certain specific regions The body build was defined on the basis of height femoral condylar breadth and the ratio of those two factors termed the skeletal quotient Subjects with a high skeletal quotient thus had light skeletons in relation to body height, and those with a low skeletal quotient heavy skeletons in relation to height. Also determined were the total hemoglobin using the alveolar CO method and the hemoglobin concentration by spectrophotometry Lastly the heart volume was determined with the subjects prone

The total investigation comprised several different subject groups Six hundred and thirty three conscripts 18 years of age were examined on registration for military service On induction a group of 178 randomly chosen conscripts were examined in addition to another group of 88 who had been selected at the time of registration This group of 88 plus a randomly chosen series of 204 were subsequently examined during military service —A further group consisting of 63 naval cadets aged 20 were examined in connection with their aptitude tests for enrolment at the Royal Swedish Naval College (KSS) and again after three months training —Yet another series comprising 48 officers and warrant officers (GIS trainees) who were attending the Swedish Armed Physical Training School and who had a mean age of 30 were examined at the start of a training course, then again after one month and after four months of physical training —Lastly similar examinations were carried out in a series of athletes—one group comprising 48 weight lifters and wrestlers with a mean age of 26 and the other 38 runners, also with a mean age of 26 In all a total of 1533 subjects were studied

The results showed an increase of height until the age of approximately 21 For the conscripts a significant increase of 1.3 cm was noted as from the time of registration

Acknowledgements

This investigation was carried out at the Military Medical Examination Centre which is under the admi nistration of th Swedish Defence Medical Board and conducts its work at Karolinska Sjukhuset in conjunction with the Department of Cli nical Physiology

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special selections with respect to physique and who had pursued specialized forms of training Since the runners training had principally involved the circulatory organs it is here termed »primary circulatory exercise» The training of the weight lifters and wrestlers on the other hand had developed chiefly the muscular strength and is thus designated as »primary muscular exercise»

Neither in the conscripts and naval cadets nor in the GIS trainees were there any conspicuous disparities in skeletal type and body weight between those with the greatest and those with the least increase of PWC. However those who showed the greatest increases in PWC and muscular strength had the lowest initial values

Although the runners had the highest PWC values their muscular strength was relatively modest. Development primarily of the physical working capacity thus does not necessarily lead to augmentation of the muscular strength—The weight lifters and wrestlers furthermore exhibited the highest muscular strength values, yet their PWC was largely equivalent to that recorded at initial examination of the GIS trainees Thus training which principally aims at development of the muscular strength will not necessarily result in a commensurate improvement of the physical working capacity

Both among the conscripts and among the naval cadets the short light skeletoned subgroups had the highest values for PWC per kilogram of body weight (relative PWC) whereas the tall heavy skeletoned subgroups showed the highest absolute values for PWC and muscular strength Further the weight lifters and wrestlers had heavy skeletons in relation to height as well as a high muscular strength whereas the runners had light skeletons in relation to height as well as a high physical working capacity In view of these findings the question arises whether persons with light skeletons in relation to height are not better suited than others for duties in which both a high absolute PWC and a high relative PWC are important considerations By the same token it is questioned whether individuals with heavy skeletons in relation to height are not better equipped than others for duties which make high demands on the absolute PWC as well as on the muscular strength If these questions can be answered in the affirmative then persons with an ordinary skeletal quotient should be best fitted for duties requiring not only high absolute and relative physical working capacities but also a high degree of muscular strength

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TABLES

Table 1 C libraflons of the cycle ergometers

Calibration no	Cycle no. 1		Cycle no. 2		Cycle no. 3		Cycle no. 4	
	SD_{Diff}	V_M	SD_{Diff}	V_M	SD_{Diff}	V_M	SD_{Diff}	V_M
I	41.1	2.8	19.3	1.3	32.9	2.3	45 6	3.1
II	11.7	0 8	23.9	1.6	28.5	1.9	26.3	1 8
III	20 6	1.5	39.1	2.6	23.2	1.7	13 4	0.9
IV	13 1	0.9	45 8	3.0	16.3	1 1	14.9	1.0
V	3.9	0 3	—	—	—	—	—	—

SD_{Diff} = Standard deviation of the differences betwe en tw consecutiv calibrations
V_M = SD_{Diff} in per cent of M for tw calibrations

Table 2 Conscripts at the registrations of 1957—1960

Determination	Year f registration															
	1957				1958				1959				1960			
		M	SD	μ		M	SD	μ	n	M	SD	μ		M	SD	μ
Height, cm	320	178 9 (178.8)	6 6	0.4	170	177 7 (176.1)	6 0	0.5	70	177.3 (176.3)	6.4	0.8	73	177 9	6 9	0 8
Weight, kg	320	68.2 (64.7)	8.2	0 5	170	66.0 (64 9)	7 4	0.6	70	67 1 (64.9)	6.9	0 8	73	67 8	8.4	1.0
Shoulder thrust, kp	—	—	—	—	170	61 9	8.7	0 7	70	61.0	9.2	1 1	—	—	—	—
pull, kp	—	—	—	—	170	37 7	7 4	0 6	70	41.1	8.3	0 8	—	—	—	—
Right hand-grip, kp	—	—	—	—	170	42.3	5 4	0 4	70	41 8	5 4	0 7	—	—	—	—
Left kp	—	—	—	—	170	39 1	5 6	0 4	70	38.3	4.5	0.6	—	—	—	—
PWC	320	918	166	10	170	892	164	13	70	881	244	29	73	970	187	22
Rel PWC	320	13.9	2.4	0.1	170	13.6	2 4	0.2	70	13.1	3 5	0 4	73	13 7	2.6	0.3
Heart olume, ml	289	768	111	7	161	783	102	8	70	762	94	11	73	771	102	12
Pulse resting, beats/min	320	73.3	12 5	0.8	170	75 4	12 1	0.9	70	77 8	15.7	1.9	73	75.3	11.5	1.3
standing	320	94 8	16.5	0 9	170	98 6	17.0	1 3	70	96 4	14.4	1.8	73	96.7	14 6	1 7

Tabl 3 Conscripts examined on registration and induction and several times during military service

Determination	Registration		Induction		Military service							
	Autumn 1957		Spring 1958		Aug 1958		Nov 1958		Jan 1959		March 1959	
		M		M		M		M		M		M
Age ears	27	18 0	27	18 5	27	18 7	27	19 0	27	19 2	27	19.3
Height cm	27	175 6	27	176 2	27	176 9	27	178.8	27	176 9	27	177.3
Weight, kg	27	63.4	27	65.7	27	66.3	27	67.3	27	66 5	27	66.7
Shoulder thrust, kp		—	—	—	—	—	27	62 6	27	63 4	27	63.4
PWC	27	919	27	1 049	27	1 194	27	1 197	27	1 131	27	1 178
Rel. PWC	27	14.1	27	16.0	27	18 0	27	17.8	27	17.0	27	17.6
Heart volume ml	27	736	27	770	27	816	27	837	27	809	27	833
Pulse resting, beats/min	27	75 2	27	70 6	27	65 6	27	63.8	27	66 5	27	64.6
standing	27	93 4	27	91 1	27	83 6	27	84.0	27	87.8	27	83.9

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Table 6 All conscripts examined on registration and induction, and during military service

Determination	I Registration		II Induction		III Mil. service		II—I		III—II	
	n	M	n	M	n	M	Diff	P	Diff	P
Height, cm	88	176.0	88	177.3	88	177 7	1.3	xxx	0.4	xx
Femur cond. breadth, cm	—	—	88	9 76	88	9 76	—	—	0	> 0.2
Skeletal quotient	—	—	88	18.2	88	18.3	—	—	0.1	> 0.2
Weight, kg	83	66.2	88	67.6	88	67.8	1.4	xxx	0.2	> 0.1
Shoulder thrust, kp	—	—	83	65.0	82	66 7	—	—	1.7	> 0.2
pull, kp	—	—	54	43.9	52	45 3	—	—	1.6	> 0.2
Right hand-grip, kp	—	—	84	44.2	83	45 0	—	—	0.8	> 0.2
Left kp	—	—	84	41 1	83	41.4	—	—	0.3	> 0.2
Skinfold thickness, mm										
region I	—	—	88	8 90	88	8.36	—	—	0.54	> 0.2
region II	—	—	88	7 76	88	7.05	—	—	0.71	> 0.2
region III	—	—	88	8.36	88	7.87	—	—	0 49	> 0.2
PWC	88	930	88	1 033	88	1 156	103	xxx	123	xxx
Rel. PWC	88	14.2	88	15.4	88	17 1	1.2	xxx	1 7	xxx
Heart volume, ml	88	765	88	783	88	815	28	xxx	32	xxx
Pulse, resting, beats/min	88	73.4	88	69.7	88	64.6	3.7	xx	5.1	xxx
standing	88	94 4	88	90.7	88	85.8	3.7	xx	4.9	xx
Blood pressure, mm Hg										
systolic	—	—	88	127.9	88	125.9	—	—	2.0	> 0.1
diastolic	—	—	88	76 8	88	72.7	—	—	4.1	xxx
Hb concentration, g/100 cm2	88	14.32	88	14 41	88	14 13	0 09	>0.2	0.28	> 0.2

Table 7 Conscripts whose physical working capacity increased most and least, respectively between registration and induction

Determination	n	%	A	n	%	B	Diff	P
Height, cm	34	39	176.8	36	41	175.2	1.6	> 0.2
Weight, cm	34	39	67.3	36	41	64.6	2.7	> 0.2
PWC on registration	34	39	852	36	41	962	100	xx
induction	34	39	1 066	36	41	927	139	xxx
Rel. PWC on registration	34	39	12.7	36	41	14.8	2.1	xxx

A = Those whose physical working capacity increased by more than 100 kpm/min
B = less 100

Table 8 Conscripts whose physical working capacity increased most and least, respectively between induction and the first examination during military service

Determination	n	%	A		%	B	Diff	P
Height, cm	40	48	177.2	28	32	176 6	0 6	> 0.2
Femur cond. breadth, cm	40	48	9 76	28	32	9.83	0.07	> 0.2
Weight, kg	40	48	67.6	28	32	68.1	0.8	> 0.2
PWC on induction	40	48	981	28	32	1 104	123	xx
PWC during mil. service	40	48	1 230	28	32	1 056	174	xxx
Rel. PWC on induction	40	48	14.7	28	32	16.2	1.5	xx

A = Those whose physical working capacity increased by more than 100 kpm/min
B = less 100

Table 4 Conscripts examined on registration and induction and during military service

Determination	Registration		Induction		Mil. service	
	Autumn 1957		Spring 1959		Aug 1959	
	n	M	n	M	n	M
Age years	47	18 0	47	19.5	47	19 7
Height, cm	47	176 9	47	178.6	4	178.7
Weight, kg	47	66.3	47	68.6	4	68.5
Shoulder thrust kp	—	—	40	66.5	46	66 7
PWC	47	929	47	1 018	47	1 152
Rel. PWC	47	14.1	47	14 9	47	16 6
Heart volume, ml	47	743	47	784	47	811
Pulse resting, beat/min	47	73 9	47	0 7	47	65 8
» standing, »	47	91 9	47	91.6	4	86.3

Table 5 Conscripts examined on registration and induction and during military service

Determination	Registration		Induction		Mil. service	
	Autumn 1958		Spring 1959		Aug 1959	
	n	M	n	M	n	M
Age years	14	18.0	14	18 5	14	18.7
Height, cm	14	176 1	14	176 7	14	176 9
Weight, kg	14	64 7	14	65 6	14	66 1
Shoulder thrust, kp	13	57.3	13	59 0	13	62 7
PWC	14	964	14	1 054	14	1 169
Rel. PWC	14	15 0	14	16 2	14	17 9
Heart volume ml	14	89	14	773	14	781
Pulse, resting, beats/min	14	72 6	14	65 7	14	64.0
standing,	14	82.5	14	80 7	14	69 9

Table 11 Relationship of body parameters to physical working capacity [illegible] conscripts

Time of examination	n	x	y	r_{xy}	P	Equation of regression line	s_{xy}
Registration	88	Heart volume	PWC	0.53	xxx	$y = 0.88x + 373$	136
	88	Height	»	0.34	xx	$y = 8.97x - 648$	151
	88	Weight		0.33	xx	$y = 6.80x + 475$	152
Induction	88	Heart volume		0.57	xxx	$y = 1.01 + 232$	139
	88	Height		0.29	xx	$y = 8.22x - 424$	161
	88	Femur cond.breadth		0.40	xxx	$y = 166.5x - 602$	154
	88	Skeletal quotient		−0.14	>0.1	$y = 1\,671 - 29.6x$	166
	88	Weight		0.39	xxx	$y = 8.21 + 478$	155
Mil. service	88	Heart volume		0.56	xxx	$y = 0.99x + 346$	139
	88	Height		0.26		$y = 7.09x - 212$	165
	88	Femur cond.breadth		0.35	xxx	$y = 140x - 268$	159
	88	Skeletal quotient		−0.15	>0.1	$y = 1\,757 - 32.9x$	168
	68	Weight		0.41	xxx	$y = 9.82x + 489$	166

Table 12 Relationship [illegible] body parameters to physical working capacity of conscripts

Time of examination	n	x	y	r_{xy}	P	Equation of regression line	s_{xy}
Induction	53	Height	Shoulder thrust	0.17	>0.2	$y = 0.33x + 6.4$	11.4
	53	Femur cond.breadth		0.15	>0.2	$y = 4.07x + 25.2$	11.4
	53	Skeletal quotient		−0.02	>0.2	$y = 60.85 - 0.37x$	11.5
	53	Weight		0.55	xxx	$y = 0.9x + 4.2$	9.7
Mil. service	52	Height		0.22	x	$y = 0.35 + 1.65$	9.5
	52	Femur cond breadth		0.24		$y = 8.74x + 8.64$	9.4
	52	Skeletal quotient		−0.09	>0.2	$y = 84.73 - 1.08x$	9.7
	52	Weight		0.38	xxx	$y = 0.52x + 20.4$	9.0

Table 9 Conscripts whose muscular strength increased most and least respectively between induction and the first examination during military service

Determination	n	%	A	n	%	B	Diff	P
Height, cm	24	39	178.4	23	38	178 6	0.2	> 0.2
Femur cond. breadth cm	24	39	9 78	23	38	9.80	0 02	> 0.2
Weight, kg	24	39	6 3	23	38	69.8	2.5	> 0.2
PWC on induction	24	39	1 021	23	38	1 041	20	> 0.2
Rel. PWC on induction	24	39	15.2	23	38	15 0	0.2	> 0.2
Shoulder thrust on induction, kp	24	39	59 5	23	38	70 7	11.2	xxx
Shoulder thrust during mil. service kp	24	39	67 1	23	38	66.6	0 5	> 0.2

A = Those whose muscular strength increased most
B = » » » least

Table 10 Conscripts subgrouped in four categories on the basis of skeletal type

Determination	Category: Tall, heavy-skeletoned						Tall, light-skeletoned					
	Registration		Induction		Mil. service		Registration		Induction		Mil. service	
	n	M	n	M	n	M	n	M	n	M	n	M
Height, cm	22	178 9	22	179.8	22	180 4	22	182 0	22	183 9	22	183 8
Femur cond. breadth cm	—	—	22	10 22	22	10 16	—	—	22	9 73	22	9 75
Skeletal quotient	—	—	22	17 6	22	17.8	—	—	22	19 0	22	18.9
Weight kg	22	72 4	22	74 0	22	73 7	22	67 7	22	69 7	22	70.1
Shoulder thrust kp	—	—	11	69 2	11	71 4	—	—	16	66 9	16	67 8
Skinfold thickness region I mm	—	—	22	8 62	22	8 86	—	—	22	7 64	22	7.83
PWC	22	1 011	22	1 102	22	1 200	22	945	22	1 064	22	1 186
Rel. PWC	22	14 1	22	15 1	22	16 4	22	14 1	22	15 3	22	16.9
Heart volume ml	22	827	22	859	22	850	22	752	22	788	22	820
Pulse resting, beats/min	22	70 9	22	66 8	22	63 0	22	71 1	22	71 8	22	65 1
» standing »	22	93 0	22	89 2	22	85 9	22	95.0	22	91 7	22	83 3

Determination	Category: Short, heavy-skeletoned						Short, light-skeletoned					
	Registration		Induction		Mil. service		Registration		Induction		Mil. service	
	n	M	n	M	n	M	n	M	n	M		M
Height, cm	22	168 9	22	170.3	22	170 8	22	172.8	22	173 4	22	175 5
Femur cond. breadth, cm	—	—	22	9 75	22	9 74	—	—	22	9 40	22	9 40
Skeletal quotient	—	—	22	17 5	22	17 6	—	—	22	18.7	22	18 7
Weight, kg	22	67 3	22	63.9	22	64.2	22	62.1	22	62.6	22	64 0
Shoulder thrust, kp		—	12	60 3	12	63 0	—	—	14	62 6	12	64.0
Skinfold thickness region I mm	—	—	22	9 01	22	9.18	—	—	22	8 45	22	7.81
PWC	22	856	22	980	22	1 109	22	907	22	969	22	1 136
Rel. PWC	22	13.8	22	15 4	22	17.2	22	14 7	22	15.8	22	17.5
Heart volume, ml	22	724	22	764	22	797	22	723	22	725	22	779
Pulse resting, beats/min.	22	78.8	22	72.4	22	65.6	22	73.1	22	68.0	22	61 3
standing	22	97 9	22	92.2	22	86.8	22	89 1	22	88.7	22	82.1

Table 11 Relationship [illegible] body parameters to physical working capacity of conscripts

Time of examination	n	x	y	r_{xy}	P	Equation of regression line	s_y
Registration	[illegible]	Heart volume	PWC	0.[illegible]	xxx	y = 0.88x + 273	1[illegible]
	[illegible]	Height		0.34	xx	y = [illegible].97 — 6[illegible]	151
	[illegible]	Weight		[illegible]	xx	y = [illegible]x + 47[illegible]	15[illegible]
Induction	[illegible]	Heart volume		0.57	xxx	y = 1 01 + [illegible]	1[illegible]
	[illegible]	Height		0 [illegible]	xx	y = 8.[illegible]x — 4[illegible]	161
	[illegible]	Femur cond breadth		0.40	xxx	y = 166.5x — 5[illegible]	1[illegible]
	[illegible]	Skeletal quotient		—0.14	>0 1	y = 1 [illegible]71 — [illegible]x	16[illegible]
	[illegible]	Weight		0.[illegible]	xxx	y = 8.21x + 478	1[illegible]
Mil. service	[illegible]	Heart [illegible]		[illegible]	xxx	y = 0 [illegible]x + [illegible]	1[illegible]
	[illegible]	Height		0.26		y = 7.[illegible]x — 212	165
	[illegible]	Femur cond breadth		0.[illegible]	xxx	y = 146x — 268	169
	[illegible]	Skeletal quotient		—0.15	>0.1	y = 1 7[illegible]7 — [illegible].9x	16[illegible]
	[illegible]	Weight		0 41	xxx	y = 9.[illegible]x + 4[illegible]	1[illegible]

Table 1[illegible] Relationship [illegible] body parameters to physical working capacity [illegible] conscripts

Time of examination		x	y	r_{xy}	P	Equation of regression line	s_y
Induction	63	Height	Shoulder thrust	[illegible].17	>0.2	y = 0.[illegible]x + [illegible].4	11 4
	63	Femur cond.breadth		0 15	>0.2	y = 4.07 + 25.[illegible]	11.4
	63	Skeletal quotient		—0.0[illegible]	>0.2	y = [illegible].85 — 0.27	11.[illegible]
	63	Weight		0 [illegible]	xxx	y = 0 [illegible]x + 4.2	9 7
Mil. service	62	Height		0.[illegible]		y = 0.25 + 1.[illegible]	[illegible]
	62	Femur cond.breadth		0 24		y = [illegible].74x + [illegible]	[illegible].4
	52	Skeletal quotient		—0.09	>0.2	y = 84.73 — 1.08x	[illegible].7
	62	Weight		0 [illegible]	xxx	y = 0.[illegible]x + 2[illegible].4	[illegible]

Table 13 Values determined on induction in two different groups of conscripts

Determination	Group 1				Group 2				Diff	P
	n	M	SD	ϵ_M	n	M	SD	ϵ_M		
Height, cm	178	178.4	6.4	0.6	88	177.3	6.0	0.6	1.1	> 0.1
Femur cond. breadth cm	178	9.84	0.43	0.03	88	9.78	0.40	0.04	0.06	> 0.2
Skeletal quotient	178	18.3	0.73	0.05	88	18.2	0.8	0.1	0	> 0.2
Weight, kg	178	67.7	8.3	0.6	88	67.6	7.9	0.8	0.1	> 0.2
Shoulder thrust kp	178	57.8	10.1	0.8	53	65.0	11.4	1.6	7.2	xxx
» pull, kp	178	42.3	7	0.5	54	43.9	7.9	1.1	1.6	> 0.2
Right hand-grip kp	178	43.0	5.4	0.4	54	44.2	5.7	0.8	1.2	> 0.2
Left » kp	178	40.0	5.8	0.4	54	41.1	6.9	0.9	1.1	> 0.2
Skinfold thickness, mm										
region I	178	8.00	3.60	0.27	88	8.90	3.9	0.4	0.9	> 0.2
region II	178	6.70	4.32	0.32	88	7 6	5.0	0.5	1.06	> 0.2
region III	178	8.32	4.88	0.37	88	8.36	4.6	0.5	0.04	> 0.2
PWC	178	1 037	196	15	88	1 033	167	18	4	> 0.2
Rel. PWC	178	15.4	2.5	0.2	88	15.4	2.4	0.3	0	> 0.2
Heart volume ml	178	783	117	9	88	783	95	11	0	> 0.2
Pulse resting, beats/min	178	73.2	10.9	0.8	88	69.7	9.5	1.0	3.5	> 0.2
» standing, »	178	92.6	13.2	1.0	88	90.7	11.6	1.3	1.9	> 0.2
Blood pressure, mm Hg										
systolic	178	127.8	9.8	0.8	88	127.9	9.9	1.1	0.1	> 0.2
diastolic	178	77.2	6.3	0.5	88	78.8	9.1	1.0	0.4	> 0.2
Hb concentration, g/100 ml	178	14.19	0.8	0.07	88	14.41	0.81	0.09	0.22	> 0.2

Group 1 = Conscripts selected on induction
Group 2 = » registration

Table 14 Values determined during military service in two different groups of conscripts

Determination	Group 1				Group 2				Diff	P
	n	M	SD	ε_M	n	M	SD	ε_M		
Height, cm	204	177.6	6.4	0.5	88	177.7	5.8	0.6	0.1	> 0.2
Femur cond. breadth, cm	204	9.80	0.45	0.03	88	9.76	0.41	0.04	0.04	> 0.2
Skeletal quotient	204	18.3	0.8	0.06	88	18.3	0.8	0.1	0.1	> 0.2
Weight, kg	204	68.0	7.7	0.5	88	67.8	7.0	0.8	0.2	> 0.2
Shoulder thrust, kp	204	63.0	9.5	0.7	52	65.7	9.7	1.0	2.7	> 0.1
pull, kp	204	43.1	7.6	0.5	52	42.3	7.5	0.8	0.8	> 0.2
Right hand-grip, kp	204	44.0	6.1	0.4	52	45.0	6.0	0.6	1.0	> 0.2
Left kp	204	40.8	6.1	0.4	53	41.4	6.5	0.7	0.6	> 0.2
Skinfold thickness, mm										
region I	204	7.29	2.00	0.14	88	8.36	3.1	0.3	1.07	xxx
region II	204	5.85	2.47	0.17	88	7.05	3.4	0.4	1.20	xxx
region III	204	6.54	2.77	0.19	88	7.87	3.8	0.4	1.33	xx
PWC	204	1 162	178	12	88	1 155	170	18	7	> 0.3
Rel. PWC	204	17.2	2.8	0.2	88	17.1	2.4	0.3	0.1	> 0.3
Heart volume, ml	204	818	106	7.4	88	815	94	10	3	> 0.2
Pulse, resting, beats/min	204	66.3	10.2	0.7	88	64.6	9.3	1.0	1.7	> 0.1
standing	204	82.9	12.3	0.9	88	85.8	10.6	1.1	2.9	> 0.1
Blood pressure, mm Hg										
systolic	204	131.4	8.5	0.6	88	125.9	8.7	0.9	5.5	xxx
diastolic	204	75.6	7.4	0.5	88	72.7	8.2	0.9	2.9	> 0.3
Hb concentration, g/100 ml	204	13.87	0.83	0.06	88	14.13	0.76	0.08	0.26	> 0.3

Group 1 = Conscripts selected after six months of military service
Group 2 = … on registration

Table 15 Values determined on induction and during military service respectively in two different groups of conscripts

Determination	Induction		Mil. service		Diff	P
	n	*M*	*n*	*M*		
Height cm	178	1 8 4	204	177.6	0.8	> 0.2
Femur cond. breadth, cm	178	9.84	204	9 80	0.04	> 0.2
Skeletal quotient	178	18.2	204	18 2	0	> 0.2
Weight, kg	178	67 7	204	68.0	0.3	> 0.2
Shoulder thrust, kp	1 8	57 8	204	63 0	5.2	xxx
» pull, kp	178	42.3	204	43.1	0 8	> 0.2
Right hand-grip kp	178	43 0	204	44 0	1.0	> 0.1
Left » » kp	178	40.0	204	40.8	0.8	> 0.1
Skinfold thickness mm						
region I	178	8.00	204	7.29	0 71	x
region II	178	6.70	204	5 85	0.85	x
region III	178	8.32	204	6.54	1 78	xx
PWC	178	1 03	204	1 162	125	xxx
Rel. PWC	178	15 4	204	1 .2	1 8	xxx
Heart volume ml	178	783	204	818	35	xx
Pulse, resting, beats/min	178	73.2	204	66.3	6.9	xxx
» standing, »	178	92.6	204	82.9	9 7	xxx
Blood pressure, mm Hg						
systolic	178	127.8	204	131 4	3 6	xxx
diastolic	178	77.2	204	75 6	1 6	x
Hb concentration, g/100 ml	178	14 19	204	13 87	0.32	> 0.2

Induction Conscripts selected on induction
Mil. service » » after six months of military service

Table 16 Conscripts subgrouped in four categories on the basis of skeletal type

C t g y

Determination	Tall, heavy-skeletoned				Tall, light-skeletoned			
	Induction		Mil. service		Induction		Mil. service	
	n	M		M		M		M
Height, cm	45	181 1	49	181.2	47	184.9	50	184 8
Femur cond. breadth, cm	45	10.27	49	10.26	47	9 88	50	9 78
Skeletal quotient	45	17 7	49	17 7	47	18 8	50	18.9
Weight, kg	45	72.8	49	74 4	47	70.2	50	69.9
Shoulder thrust, kp	45	61 4	49	67.1	47	54.7	50	61.2
Skinfold thickness region 1 mm	45	8 64	49	7 96	47	7 62	50	6 66
PWC	45	1 123	49	1 204	47	1 030	50	1 190
Rel. PWC	45	16 8	49	16.2	47	14 5	50	17 1
Heart volume ml	45	838	49	877	47	815	50	838
Pulse, resting, beats/min	45	71.1	49	63 9	47	74.4	50	68 6
standing	45	91 8	49	84 6	47	97.2	50	87.2

C a t g y

Determination	Short, heavy-skeletoned				Short, light-skeletoned			
	Induction		Mil. service		Induction		Mil. service	
		M		M		M		M
Height, cm	44	171 4	53	171.3	43	175.8	52	173.8
Femur cond. breadth, cm	44	9.82	53	9 83	42	9.37	52	9 34
Skeletal quotient	44	17.5	53	17.6	42	18 7	53	18.8
Weight, kg	44	65.4	53	66 7	42	62 0	52	62.7
Shoulder thrust, kp	44	59 6	53	63.7	42	55 6	52	60 1
Skinfold thickness region 1, mm	44	8.67	53	7 58	42	7 14	52	6.96
PWC	44	1 048	53	1 146	42	964	52	1 112
Rel. PWC	44	16 1	53	17.5	42	15 4	52	17.8
Heart volume ml	44	780	53	792	42	720	52	767
Pulse resting, beats/min	44	73 9	53	65.7	42	73.4	52	65.0
standing	44	89 4	53	80.3	42	92 0	52	78.7

Table 17 Relationship of body parameters to physical working capacity in conscripts selected respectively on induction and during military service

Time of examination	n	x	y	r_{xy}	P	Equation of regression line	e_{xy}
Induction	178	Heart volume	PWC	0.64	xxx	y = 0.90x + 328	167
»	178	Height	»	0.19	xx	y = 5.97x — 29	193
»	178	Femur cond.breadth	»	0.40	xxx	y = 160.1x — 736	181
»	178	Skeletal quotient	»	—0.26	xxx	y = 2 284 — 68.7x	190
»	178	Weight	»	0.48	xxx	y = 11.4x + 263	172
Mil. service	204	Heart volume	»	0.51	xxx	y = 0.96x + 457	154
»	204	Height	»	0.34	xxx	y = 9.48x — 622	168
»	204	Femur cond.breadth	»	0.34	xxx	y = 132.9x — 140	168
»	204	Skeletal quotient	»	—0.01	> 0.2	y = 1 161 — 1.96x	179
»	204	Weight	»	0.41	xxx	y = 9.3x + 627	165

Table 18 Relationship of body parameters to muscular strength (shoulder thrust) in conscripts selected respectively on induction and during military service

Time of examination	n	x	y	r_{xy}	P	Equation of regression line	e_{xy}
Induction	178	Height	Shoulder thrust	— 0.04	> 0.2	y = 70.3 — 0.07x	10.2
»	178	Femur cond.breadth	»	0.24	xx	y = 5.66x + 2.11	9.9
»	178	Skeletal quotient		—0.31	xxx	y = 184.6 — 4.2x	9.7
»	178	Weight	»	0.34	xxx	y = 0.41x + 30	9.6
Mil. service	204	Height	»	0.16	x	y = 0.234x + 21.5	9.4
»	204	Femur cond.breadth	»	0.44	xxx	y = 9.2x — 27.0	8.5
»	204	Skeletal quotient	»	— 0.35	xxx	y = 140.6 — 4.27	8.9
»	204	Weight		0.53	xxx	y = 0.65x + 18.7	8.0

Table 19 Naval cadets before and after three months of physical training

Determination		May				Aug			Diff	P
		M	SD	m		M	SD	m		
Height, cm	63	170.4	6.2	0.8	63	170.8	6.2	0.8	0.4	xxx
Femur cond. breadth, cm	63	9.72	0.43	0.05	63	9.76	0.45	0.06	0.03	> 0.2
Skeletal quotient	63	18.4	0.71	0.09	63	18.4	0.81	0.10	0	> 0.2
Weight, kg	63	68.6	8.0	1.0	63	69.6	7.1	0.9	1.0	xx
Shoulder thrust, kp	63	63.9	8.8	1.1	63	69.1	8.8	1.1	5.2	xx
pull, kp	63	42.6	8.4	1.1	63	45.3	7.9	1.0	2.7	> 0.1
Right hand grip, kp	63	43.8	6.4	0.8	63	45.4	6.9	0.7	1.6	> 0.2
Left kp	63	40.9	5.8	0.7	63	41.7	5.6	0.7	0.8	> 0.2
Skinfold thickness, mm										
region I	63	8.67	2.07	0.26	63	7.86	1.85	0.23	0.81	> 0.1
region II	63	7.41	2.06	0.26	63	6.41	2.11	0.27	1.00	
region III	63	9.02	2.63	0.46	63	7.13	2.33	0.29	1.89	xxx
PWC	63	984	192	24	63	1 112	175	22	127	xxx
Rel PWC	63	14.4	2.5	0.3	63	16.1	2.6	0.3	1.7	xxx
Heart volume, ml	63	782	97	12	63	804	107	14	22	xxx
Pulse, resting, beats/min	63	70.6	10.4	1.3	63	66.6	9.3	1.2	4.0	
standing	63	88.6	13.0	1.6	63	85.8	12.4	1.2	2.8	> 0.2
Blood pressure, mm Hg										
systolic	63	129.2	8.7	1.1	63	133.7	10.0	1.3	3.6	xx
diastolic	63	76.2	8.0	1.0	63	72.1	9.1	1.2	4.1	xx
Hb concentration, g/100 ml	63	14.62	0.98	0.12	63	14.14	0.89	0.11	0.48	

Table 20 Naval cadets whose physical working capacity increased most and least, respectively during three months of physical training

Determination		%	A	n	%	B	Diff	P
Height, cm	27	43	178.5	21	33	179.9	1.4	> 0.2
Femur cond. breadth, cm	27	43	9.74	21	33	9.75	0.01	> 0.2
Weight, kg	27	43	66.4	21	33	71.1	4.8	
PWC in May	27	43	896	21	33	1 088	192	xxx
PWC in Aug	27	43	1 141	21	33	1 067	104	xx
Rel. PWC in May	27	43	13.6	21	33	16.4	1.8	

A = Those whose physical working capacity increased by more than 100 kpm/min
B = less 100

Table 1 Naval cadets whose muscular strength increased most and least respectively during three months of physical training

Determination	n	%	A	n	%	B	Diff	P
Height, cm	28	44	178.5	26	41	180 7	2.2	>0.1
Femur cond. breadth cm	28	44	9 75	26	41	9 78	0 03	>0.2
Weight kg	28	44	67 9	26	41	70.9	3.0	>0.2
PWC in May	28	44	971	26	41	1 040	69	>0.2
Rel. PWC in May	28	44	14 4	26	41	14 8	0 4	>0.2
Shoulder thrust in May kp	28	44	61.5	26	41	66.1	4.3	>0 1
Aug, kp	28	44	71.2	26	41	68 7	2.5	>0 2

A = Those whose muscular strength increased most
B = » » » » least

Table Naval cadets subgrouped in four categories on the basis of skeletal type

Determination	Category							
	Tall, heavy skeletoned				Tall, light-skeletoned			
	May		Aug		May		Aug	
	n	M	n	M	n	M	n	M
Height, cm	18	181.5	18	181 8	15	186.0	15	186.6
Femur cond. breadth cm	18	10 12	18	10 16	15	9 79	15	9 77
Skeletal qoutient	18	17 9	18	17 9	15	19 0	15	19 1
Weight, kg	18	73.2	18	73.0	15	70 0	15	72.4
Shoulder thrust, kp	18	66.7	18	71 0	15	65.5	15	70 5
Skinfold thickness region III mm	18	9 4	18	6.6	15	8.9	15	7 4
PWC	18	975	18	1 133	15	993	15	1 120
Rel. PWC	18	13.4	18	15 7	15	14 2	15	15.5
Heart volume ml	18	808	18	799	15	805	15	827
Pulse resting, beats/min	18	73 7	18	68 9	15	71 1	15	66.5
» standing, »	18	87 4	18	91 5	15	94 4	15	87 3

Determination	Category							
	Short, heavy-skeletoned				Short, light-skeletoned			
	May		Aug		May		Aug	
	n	M	n	M	n	M	n	M
Height, cm	15	173.0	15	178 3	15	176.6	15	177 1
Femur cond. breadth cm	15	9 67	15	9 73	15	9,28	15	9 31
Skeletal quotient	15	17 9	15	17 8	15	19 1	15	19 0
Weight, kg	15	66.1	15	67 0	15	64 3	15	65 5
Shoulder thrust, kp	15	61 3	15	67 6	15	61 6	15	67 1
Skinfold thickness region III mm	15	10.2	15	8 1	15	7 5	15	6 5
PWC	15	940	15	1 090	15	1 033	15	1 100
Rel. PWC	15	14.2	15	16.3	15	16 0	15	16.8
Heart volume, ml	15	753	15	809	15	755	15	782
Pulse resting beats/min	15	70.4	15	66.3	15	66.1	15	63.9
standing »	15	87.5	15	82.1	15	85 4	15	81 7

Table 23 Relationship of body parameters to physical working capacity of naval cadets

Time of examination		x	y	r_{xy}	P	Equation of regression line	s_{xy}
May	63	Heart volume	PWC	0.42	xx	$y = 0.79x + 867$	171
	63	Height		0.21	> 0.05	$y = 6.63x - 204$	189
	63	Femur cond.breadth		0.11	> 0.2	$y = 50.6x + 482$	193
	63	Skeletal quotient		0.07	> 0.2	$y = 18.7 + 640$	193
	63	Weight		0.41	xx	$y = 10x + 293$	176
Aug	63	Heart volume		0.26		$y = 0.45x + 784$	170
	63	Height		0.17	> 0.1	$y = 4.73x + 262$	174
	63	Femur cond.breadth		0.22	> 0.05	$y > 66.4x + 269$	172
	63	Skeletal quotient		−0.11	> 0.2	$y = 1848 - 23.5x$	176
	63	Weight		0.24	> 0.05	$y = 5.97x + 696$	171

Table 24 Relationship of body parameters to muscular strength of naval cadets

Time of examination		x	y	r_{xy}	P	Equation of regression line	s_{xy}
May	63	Height	Shoulder thrust	0.35	xx	$y = 0.5x - 25.75$	8.3
	63	Femur cond.breadth		0.35	xx	$y = 7.13x - 5.35$	8.4
	63	Skeletal quotient		−0.07	> 0.2	$y = 79.5 - 0.36x$	8.9
	63	Weight		0.46	xxx	$y = 0.31 + 29$	7.9
Aug	63	Height		0.30		$y = 0.43x - 8.73$	8.5
	63	Femur cond.breadth		0.34	xx	$y = 6.7 + 3.88$	8.4
	63	Skeletal quotient		−0.13	> 0.2	$y = 92.8 - 1.28x$	8.8
	63	Weight		0.46	xxx	$y = 0.37 + 29.0$	7.9

Table 25 Values determined in GIS trainees before and after physical training

Determination	Oct				Nov				Feb			
	n	M	SD	e_M	n	M	SD	e_M	n	M	SD	M
Height, cm	48	177 8	6.2	0 9	48	177 0	6.1	0 9	44	177 1	6.3	1 0
Femur cond. breadth cm	48	9 83	0 46	0 07	48	9 81	0 46	0.07	44	9.84	0 47	0 07
Skeletal quotient	48	18 1	0 73	0.11	48	18.1	0.8	0 12	44	18.0	0 7	0 11
Weight, kg	48	73 4	7 0	1 0	48	73.5	7 1	1 0	44	72.1	6.7	1 0
Shoulder thrust, kp	48	69 6	9 1	1.3	48	71.5	8.2	1.2	44	71 4	9.9	1 5
» pull, kp	48	43.2	6.3	0 9	48	44 9	8.2	1.2	44	46.3	6.8	1 1
Right hand-grip, kp	48	45.3	4 1	0 6	48	45 5	4 6	0 7	44	45.8	5 3	0 8
Left » » kp	48	42.3	4 1	0 6	48	40 6	4 4	0 6	44	42.6	4 9	0.8
Skinfold thickness mm												
region I	48	9 7	3.7	0.6	48	9 2	3.5	0 5	44	8.1	2.4	0.4
region II	48	8.4	3.7	0 5	48	7 6	3.3	0.5	44	6.1	2.2	0.3
region III	48	8.6	3.7	0 5	48	7.5	3 0	0 4	44	6.2	2.2	0.3
PWC	48	1 273	214	31	48	1 382	180	26	44	1 502	196	29
Rel. PWC	48	17 4	2.8	0 4	48	18 9	2.5	0 4	44	20 9	2.6	0 4
Heart volume ml	48	947	131	19	48	969	134	20	44	978	119	18
Pulse, resting beats/min	48	60 1	11 6	1 7	48	55 9	8.4	1.2	44	55 6	7 6	1 1
» standing, »	48	74 0	15.3	2.2	48	70 1	11 5	1 7	44	71.3	11 7	1.8
Blood pressure mm *Hg*												
systolic	48	128.3	10 0	1 4	48	131.3	7 6	1 1	44	129 7	10.2	1.5
diastolic	48	81 7	7 1	1 0	48	78 4	8.0	1.2	44	77 7	9 4	1 4
Hb concentration, g/100 ml	48	13.83	0 93	0 14	48	13.50	0 91	0.14	44	14.01	0 63	0.09

Table 26 Differences for GIS trainees after one month and four months of physical training

Determination	Oct M	Nov M	Feb M	Nov—Oct Diff	p	Feb—Oct Diff	p
Height cm	177.3	177 0	177 1	0.3	> 0.2	0.2	> 0.2
Femur cond breadth cm	*9 83*	*9 81*	*9 84*	*0 02*	> 0 2	0 01	> 0 2
Skeletal quotient	18.1	18.1	18.0	0	> 0.2	0 1	> 0.2
Weight, kg	73.4	73.5	72.1	0 1	> 0.2	1 3	xx
Shoulder thrust, kp	69 6	71 5	71 4	1 9	> 0.2	1 8	> 0.2
» pull, kp	43.2	44 9	46.3	1 7	> 0.2	3.1	x
Right hand grip kp	45.3	45 5	45 8	0.2	> 0 2	0.5	> 0.2
Left » » kp	42.3	40 6	42.6	1 7	x	0 3	> 0.2
Skinfold thickness, mm							
region I	9 67	9.23	8.06	0.44	> 0.2	1 62	x
region II	8 40	7 55	6.09	0.85	> 0.2	2.31	xxx
region III	8.58	7 49	6.20	1 09	> 0 1	2.38	xxx
PWC	1 273	*1 382*	*1 502*	109	x	229	xxx
Rel. PWC	17 4	18.9	20 9	1 5	xx	3 5	xxx
Heart volume, ml	947	969	978	22	xx	31	xx
Pulse, resting, beats/min	60 1	55 9	55 6	4 2	xx	4 5	xx
» standing,	74 0	70 1	71 3	3 9	x	2.7	> 0.2
Blood pressure, mm Hg							
systolic	128.3	131 3	129 7	3.0	> 0.2	1 4	> 0.2
diastolic	81 7	78.4	77 7	3.3	> 0.2	4 0	> 0.2
Hb concentration, g/100 ml	13 83	13 50	14 01	0.33	> 0.2	0 18	> 0.2

Table 27 GIS trainees whose physical working capacity had increased most and least respectively after four months of physical training

Determination	n	%	A		%	B	Diff	P
Height, cm	17	39	177.2	14	32	176.8	0.4	> 0.2
Femur cond. breadth, cm	17	39	9.85	14	32	9.86	0.01	> 0.2
Weight, kg	17	39	72.7	14	32	73.8	1.1	> 0.2
PWC in Oct	17	39	1 182	14	32	1 364	182	
Feb	17	39	1 559	14	32	1 435	124	> 0.2
Rel. PWC in Oct	17	39	16.4	14	32	18.5	2.1	

A = Those whose physical working capacity had increased by more than 200 kpm/min
B = less 200

Table 28 GIS trainees whose muscular strength had increased and decreased respectively after four months of physical training

Determination		%	A		%	B	Diff	P
Height, cm	17	39	178.2	18	41	176.5	1.7	> 0.2
Femur cond. breadth, cm	17	39	9 96	18	41	9.81	0.15	> 0.2
Weight, kg	17	39	73.8	18	41	74.2	0.4	> 0 2
PWC in Oct	17	39	1 241	18	41	1 285	44	> 0.2
Rel. PWC in Oct	17	39	17 1	18	41	17 4	0 3	> 0.2
Shoulder thrust in Oct, kp	17	39	67 1	18	41	73 3	6 2	> 0.2
Feb, kp	17	39	76.4	18	41	68.5	7 9	

A = Those whose muscular strength had increased
B decreased

Table 29 Heavy-skeletoned and light-skeletoned GIS trainees after one month and four months of physical training

Determination	Skeletal type											
	Heavy						Light					
	Oct		Nov		Feb		Oct		Nov		Feb	
		M		M		M		M		M	n	M
Height, cm	25	175 7	25	176 6	22	175.9	23	178.8	23	178.6	23	178.4
Femur cond breadth, cm	25	10.1	25	10 0	22	10.1	23	9.8	23	9 6	22	9.6
Skeletal quotient	25	17.8	25	17 5	22	17.8	23	18.7	23	18.7	22	18.8
Weight, kg	25	74 4	25	74.7	22	74 0	23	72.0	23	72.2	23	70.0
Shoulder thrust, kp	25	70.0	25	73.4	22	74.3	23	69.1	23	69.2	22	68.3
Skinfold thickness region III mm	25	9.2	25	7.8	22	6 3	23	7.9	23	7 1	23	6.9
PWC	25	1 348	25	1 448	22	1 581	23	1 193	23	1 207	22	1 438
Rel. PWC	25	18 2	25	19.5	22	21.3	23	16.7	23	16.3	22	20.6
Heart volume, ml	25	977	25	1 008	22	1 007	23	914	23	928	22	949
Pulse resting, beats min	25	67 1	25	84 8	22	83.7	23	63.8	23	88.2	22	87.6
standing	25	70 1	25	68.9	22	67 9	23	78.1	23	74.3	22	74.7

Table 30 Heavy skeletoned and light skeletoned conscripts and naval cadets before and after three months of physical training

Determination	Conscripts							
	Heavy-skeletoned				Light-skeletoned			
	May		Aug		May		Aug	
	n	M	n	M	n	M	n	M
Height, cm	44	175 1	44	175 6	44	179 6	44	179 7
Femur cond. breadth, cm	44	10 0	44	10 0	44	9 6	44	9 6
Skeletal quotient	44	17 6	44	17 7	44	18.9	44	18.8
Weight kg	44	69 0	44	68.9	44	66.3	44	67 0
Shoulder thrust, kp	44	65 0	44	64.4	44	64.9	44	65.2
Skinfold thickness region III mm	44	8 8	44	8.5	44	8.0	44	7.3
PWC	44	1 041	44	1 153	44	1 026	44	1 161
Rel. PWC	44	15.2	44	16.8	44	15 5	44	17 4
Heart volume, ml	44	810	44	837	44	756	44	799
Pulse resting, beats/min	44	69 6	44	65.3	44	69 9	44	63.2
» standing	44	90 7	44	86 4	44	90,2	44	85.2

Determination	Naval cadets							
	Heavy skeletoned				Light-skeletoned			
	May		Aug		May		Aug	
	n	M	n	M	n	M	n	M
Height, cm	33	177 6	33	177 9	30	181 3	30	181 9
Femur cond. breadth cm	33	9 9	33	10 0	30	9.5	30	9.5
Skeletal quotient	33	17 9	33	17 9	30	19 0	30	19 1
Weight, kg	33	70.0	33	70 3	30	67 1	30	69 0
Shoulder thrust, kp	33	64 3	33	69 4	30	63 6	30	68.8
Skinfold thickness region III mm	33	9 8	33	7 3	30	8.2	30	7 0
PWC	33	959	33	1 114	30	1 013	30	1 110
Rel. PWC	33	13.8	33	16.0	30	15 1	30	18 2
Heart volume, ml	33	783	33	803	30	780	30	804
Pulse resting, beats/min	33	72.2	33	67 7	30	68.6	30	65 2
» standing »	33	87.5	33	87 1	30	89 9	30	84 5

Table 31 Relationship of body parameters to physical working capacity of GIS trainees

Time of examination		x	y	xy	P	Equation of regression line	xy
Oct	48	Heart olume	PWC	0.83	xxx	y = 0.67 + 450	187
	48	Height		0.19	> 0.2	y = 6.51 + 110	215
	48	Femur cond.breadth		0.41	xx	y = 193.6 — 620	199
	48	Skeletal quotient		—0.31	x	y = 2 939 — 91.2x	208
	48	Weight		0.23		y = 10x + 837	203
Nov	48	Heart volume		0.47	xx	y = 0.625 + 776	181
	48	Height		0.15	> 0.2	y = 4.41x + 602	180
	48	Femur cond.breadth		0.38		y = 144x — 31	170
	48	Skeletal quotient		— 0.23	> 0.05	y = 2 598 — 67.2x	175
	48	Weight		0.32		y = 8.12x + 782	173
Feb	44	Heart volume		0.62	xxx	y = 0.85x + 675	170
	44	Height		0.24	> 0.1	y = 7.84x + 167	182
	44	Femur cond breadth		0.40	xx	y = 167.2x — 144	163
	44	Skeletal quotient		— 0.28	> 0.05	y = 2 765 — 70x	191
	44	Weight		0.37		y = 10.7 + 729	185

Table 32 *Relationship of body parameters to muscular strength of GIS trainees*

Time of examination			y	xy	P	Equation of regression line	xy
Oct	48	Height	Shoulder thrust	0.03	> 0.3	y = 0.042x + 61.9	9.2
	48	Femur cond breadth		0.28	> 0.05	y = 5.2x + 18.4	8.9
	48	Skeletal quotient		— 0.38	> 0.08	y = 130.3 — 3.36x	8.9
	48	Weight		0.27	> 0.08	y = 0.35x + 42.9	8.9
Nov	48	Height		0.23	> 0.1	y = 0.21 + 17.4	8.1
	48	Femur cond.breadth		0.48	xxx	y = 8.64x — 13.2	7.3
	48	Skeletal quotient		— 0.35	x	y = 140.8 — 3.83x	7.9
	48	Weight		0.42	xx	y = 0.49x + 25.6	7.6
F b	44	Height		0.23	> 0.1	y = 0.24x + 8.71	9.8
	44	Femur cond breadth		0.53	xxx	y = 10.9x — 35.9	8.5
	44	Skeletal quotient		— 0.43	xx	y = 173.3 — 6.7	9.1
	44	Weight		0.39	x	y = 0.57 + 20.4	9.3

Table 38 Weight lifters and wrestlers and runners

Determination	Weight-lifters and wrestlers				Runners				*Diff*	*P*
	n	*M*	*SD*	ϵ_M	*n*	*M*	*SD*	ϵ_M		
Height, cm	48	173.1	6.7	1 0	28	178.9	4 6	0 9	5.8	xxx
Femur cond. breadth, cm	48	9.88	0.5	0 08	28	9 64	0.4	0 07	0.24	xxx
Skeletal quotient	48	17.5	0 66	0 09	28	18.6	0 62	0.12	1 04	xxx
Weight, kg	48	74 1	11.2	1 6	28	66.3	4.6	0 9	7 8	xxx
Shoulder thrust, kp	48	76.9	10.2	1 5	28	63.6	9.8	1 9	13.3	xxx
» pull, kp	48	54.9	9.2	1.3	28	42.0	7 6	1 4	12.9	xxx
Right hand-grip, kp	48	51 0	6.9	1 0	28	42.9	5.4	1 0	8.1	xxx
Left » » kp	48	47 6	7 0	1 0	28	40.2	5.5	1 0	7 4	xxx
Skinfold thickness, mm										
region I	48	8.75	2.6	0 4	28	5 93	0 9	0.2	2.83	xxx
region II	48	6 92	4.2	0.6	28	4.21	0 7	0 1	2.71	xxx
region III	48	7 71	4.2	0.6	28	4 79	0 9	0.2	2.92	xxx
PWC	48	1 213	219	32	28	1 607	174	33	394	xxx
Rel. PWC	48	16.5	2.8	0 4	28	24 3	2.5	0.5	7.8	xxx
Heart volume, ml	48	889	130	19	28	947	80	15	58	x
Heart volume/weight	48	12.1	1.39	0.20	28	14.3	1.30	0.25	2.2	xxx
Pulse, resting, beats/min	48	64.2	12 1	1.8	28	55.3	9 0	1 7	8.9	xxx
» standing, »	48	74 7	11 1	1 6	28	68.8	10.3	1 9	5.9	xxx
Blood pressure mm Hg										
systolic	48	136.1	9 7	1 4	28	129 8	13.5	2.6	6.3	x
diastolic	48	84 0	8.0	1.2	28	78 9	7 1	1 4	5 1	xxx
Hb concentration, g/100 ml	48	14 17	0 78	0.11	28	13 62	0 81	0.16	0.55	xxx
Total-Hb, g	24	845	132	27	20	881	76	17	36	> 0.2
Total-Hb/weight	24	11 4	0.9	0.2	20	13.2	0.9	0.2	1 8	xxx
Blood volume l	24	5 98	0 8	0.2	20	6.50	0.7	0.2	0.52	x
Blood volume/weight	24	80.7	6.9	1 4	20	97 6	9 8	2.2	16.9	xxx

Table 34 Weight-lifters and wrestlers compared with heavy-skeletoned GIS trainees, and runners compared with light-skeletoned GIS trainees

Determination	Weight-lifters and wrestlers		Heavy-skeletoned GIS trainees		Diff	P
		M	*n*	*M*		
Height, cm	48	172.1	22	170.0	2.2	> 0.1
Femur cond. breadth, cm	48	9.9	22	10.1	0.2	> 0.1
Skeletal quotient	48	17.5	22	17.5	0.0	> 0.2
Weight, kg	48	74.1	22	74.0	0.1	> 0.2
Shoulder thrust, kp	48	76.9	22	74.2	2.7	> 0.2
Skinfold thickness region III, mm	48	7.7	22	6.5	1.2	> 0.2
PWC	48	1 213	22	1 561	348	xxx
Rel. PWC	48	16.5	22	21.2	4.7	xxx
Heart volume, ml	48	889	22	1 007	118	xxx
Pulse resting, beats/min	48	64.2	22	53.7	10.5	xx
standing	48	74.7	22	67.9	6.8	x

Determination	Runners		Light skeletoned GIS trainees		*Diff*	*P*
		M	*n*	*M*		
Height, cm	28	178.9	22	178.4	0.5	> 0.2
Femur cond. breadth, cm	28	9.6	22	9.6	0.0	> 0.2
Skeletal quotient	28	18.6	22	18.6	0.0	> 0.2
Weight, kg	28	66.3	22	70.0	3.7	
Shoulder thrust, kp	28	63.6	22	68.3	4.7	> 0.1
Skinfold thickness region III, mm	28	4.8	22	5.9	1.1	> 0.2
PWC	28	1 607	22	1 438	169	xxx
Rel. PWC	28	24.3	22	20.6	3.7	xxx
Heart volume, ml	28	947	22	949	2	> 0.2
Pulse resting, beats/min	28	55.3	22	57.6	2.3	> 0.2
standing	28	68.8	22	74.7	5.9	> 0.05

Table 33 Weight-lifters and wrestlers and runners

Determination	Weight-lifters and wrestlers				Runners				Diff	P
	n	M	SD	ε_M	n	M	SD	ε_M		
Height, cm	48	173.1	6.7	1.0	28	178.9	4.6	0.9	5.8	xxx
Femur cond. breadth, cm	48	9.85	0.5	0.08	28	9.61	0.4	0.07	0.24	xxx
Skeletal quotient	48	17.5	0.66	0.09	28	18.6	0.62	0.12	1.04	xxx
Weight, kg	48	74.1	11.2	1.6	28	66.3	4.5	0.9	7.8	xxx
Shoulder thrust, kp	48	76.9	10.2	1.5	28	63.6	9.8	1.9	13.3	xxx
» pull, kp	48	54.9	9.2	1.3	28	42.0	7.6	1.4	12.9	xxx
Right hand-grip, kp	48	51.0	6.9	1.0	28	42.9	5.4	1.0	8.1	xxx
Left » » kp	48	47.6	7.0	1.0	28	40.2	5.5	1.0	7.4	xxx
Skinfold thickness, mm										
region I	48	8.75	2.6	0.4	28	5.93	0.9	0.2	2.82	xxx
region II	48	6.92	4.2	0.6	28	4.21	0.7	0.1	2.71	xxx
region III	48	7.71	4.2	0.6	28	4.79	0.9	0.2	2.92	xxx
PWC	48	1 213	219	32	28	1 607	174	33	394	xxx
Rel. PWC	48	16.5	2.8	0.4	28	24.3	2.5	0.5	7.8	xxx
Heart volume, ml	48	889	130	19	28	947	80	15	58	x
Heart volume/weight	48	12.1	1.39	0.20	28	14.3	1.30	0.25	2.2	xxx
Pulse resting, beats/min	48	64.2	12.1	1.8	28	55.3	9.0	1.7	8.9	xxx
» standing, »	48	74.7	11.1	1.6	28	68.8	10.3	1.9	5.9	xxx
Blood pressure, mm Hg										
systolic	48	136.1	9.7	1.4	28	129.8	13.5	2.6	6.3	x
diastolic	48	84.0	8.0	1.2	28	78.9	7.1	1.4	5.1	xxx
Hb concentration, g/100 ml	48	14.17	0.78	0.11	28	13.62	0.81	0.16	0.55	xxx
Total Hb g	24	845	132	27	20	881	76	17	36	> 0.2
Total-Hb/weight	24	11.4	0.9	0.2	20	13.2	0.9	0.2	1.8	xxx
Blood volume, l	24	5.98	0.8	0.2	20	6.50	0.7	0.2	0.52	x
Blood volume/weight	24	80.7	6.9	1.4	20	97.6	9.6	2.3	16.9	xxx